Social factors affecting early developmental behaviour in laboratory mouse pups (Mus musculus)

Thesis

*SOCIAL FACTORS AFFECTING EARLY DEVELOPMENTAL BEHAVIOUR IN LABORATORY MOUSE PUPS (**Mus musculus**)*

By

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ABSTRACT

Experiments were conducted to establish the effect of litter size, maternal experience and the presence of the father on laboratory mouse development. Behaviour was recorded from day 3, at the earliest, to day 30, at the latest, with the minimum of disturbance to the subjects. Daily observations were made to establish the proportion of time the pups spent exhibiting certain activities. On day 30, open field tests were conducted and the pups' body weights were measured.

The main experiment involved a 2 x 2 x 2 design so that the main effects of the three factors, and their interactions, could be assessed. Although all three factors influenced pup behaviour, paternal presence was the factor with the greatest number of significant main effects. Paternal presence had five main effects and litter size had four. Maternal experience had only one main effect, but was involved in all eight of the significant interactions. It was concluded that both the presence of the father and the small litter size increased the developmental rate of mouse pups, whereas maternal experience was involved in indirect effects.

Two further experiments were conducted to investigate routes by which the father's effect might be operating. The Split Litter experiment exposed pups to the father, in the absence of the mother. The father displayed a range of caretaking activities, very similar to the mother's, and the results and general observations indicated

that the father was directly influencing offspring behaviour. The Split Cage experiment showed that the mother's caretaking behaviour was affected by the presence of the father and was associated with changed pup behaviour.

It was concluded that the father affected pups directly, mainly by providing them with thermal insulation and tactile stimulation, and also affected them via a maternal mediation route by eliciting an increased display of maternal behaviour.

DECLARATION

I declare that this thesis, composed by myself and embodying work carried out by myself, has not previously been submitted for a degree or other qualification to this or any other university or institution.

GRAHAM MITCHELL.

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Chapter One

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

In this project I chose to investigate the effects of three social factors on laboratory mouse (Mus musculus) behavioural development; namely litter size, maternal experience and the presence or absence of the father. These three factors were chosen because:

(i) my preliminary observations suggested they were likely to be important in development, both in the laboratory and in the wild.

(ii) a body of work already exists, investigating these factors in a variety of rodents. The information in laboratory mice is, however, incomplete and the three factors listed above are rarely considered in an integrated fashion. In this project I considered the factors, not only individually, but also from the point of view of their interaction effects. In addition, I investigated the routes by which some of the revealed effects were mediated.

1. The Size of the Litter

In both a laboratory and in the wild, maternal factors and pup mortality would cause the litter size to vary from litter to litter. What effect would this have on the behavioural development of the mouse?

Some information is available on the effect of litter size on both physical and behavioural development of rodent pups.

With few exceptions (Deitchman et al. 1976), most workers have found that the size of the litter affected offspring body weights. It has been observed that when pups developed in very small litters, or even on their own, death often resulted (Kumaresan et al. 1967; Leigh & Hofer 1973; Ota & Yokoyama 1967; Priestnall 1970). Above a minimum litter size, however, which differed between different studies, rodent offspring in small litters experienced larger weight gains than those in larger litters. This association between smaller litter size and greater weight gain has been noticed in guinea pigs (Cavia porcellus) (Stern & Bronner 1970), in laboratory rats (Rattus norvegicus) (Fleischer & Turkewitz 1979; Jen et al. 1978; Lore & Moyer 1973; Milkovic et al. 1976; Padmanabhan & Singh 1980; Rosen 1958; Seitz 1954; Ward & Gerall 1968; Wehmer & Jen 1978) and in laboratory mice (Brumby 1960; Eisen et al. 1977; Gates 1925; Priestnall 1972). (In order to avoid repetition of names, the scientific name of each rodent will only be given the first time a reference is made to it in each chapter. The only exceptions are the laboratory mouse and laboratory rat. Scientific names for these animals will only be given in this chapter.)

Other studies have demonstrated that body weight is not the only measure of growth and development that has been associated with litter size. Scott (1970) found that golden hamster pups (Mesocricetus auratus) developed at a faster rate behaviourally and physically, when in a litter size of two pups, compared to a litter size of eight pups. Elwood & Broom (1978) found that gerbil litters (Meriones unguiculatus) containing three pups were behaviourally more advanced than those containing either one pup or five pups. This conclusion was based on the frequency of display of certain activities and the earliest age at which activities were displayed, in the different size litters.

In addition to this, those in a litter size of three pups were heavier than the other two groups at 25 days of age. Eye opening was also retarded in the largest size group. Fleischer & Turkewitz (1979) concluded that large-litter rearing, stunted the development of rat offspring. Animals from large litters (18 to 22 pups) showed deficits in adult learning tests when compared to subjects from small litters (4 pups). Wehmer & Jen (1978) found that rats from a litter of four pups were 10 per cent longer, grew faster, and opened their eyes earlier than rats from a litter of eight pups. Padmanabhan & Singh (1980) found that when comparing rat litters of six, eight and nine pups; the offsprings' crown-rump length, tail length and patello-calcaneal length were reduced in the large litters. Litters containing 16 rats experienced stunted development including delayed eye opening and fur eruption, when compared to litters containing eight rats (Galler & Turkewitz 1975)

In laboratory mice, Priestnall (1970) found that his subjects grew faster when developing in a small litter, but the time when hair appeared and the eyes opened was not affected by litter size.

Open field tests have been used extensively by researchers in an effort to establish the effect of litter size on rodent development. Most researchers have concluded that a low ambulation score and a high defaecation score are indicators of a state of high emotionality. Using these criteria to establish emotionality, some workers have not found a connection between litter size and emotionality in laboratory rats (Broadhurst & Levine 1963) and in laboratory mice (Gervais 1978; Gervais et al. 1977; Guttman 1978).

In contrast with these findings, a small litter size has been associated with decreased emotionality in the laboratory rat (Denenberg 1963a,b; Lore & Moyer 1973; Rosen 1958; Seitz 1954; Wehmer & Jen 1978) and in the laboratory mouse (Deitchman et al. 1976; LaBarba & White 1971; Priestnall 1973b).

It would appear that provided a litter is not too small, pups from a small litter develop at a faster rate than pups from a large litter. It is possible that different litter sizes may also affect the parents' behaviour towards the pups. Such changed parental behaviour may also help to determine the litter-size effects already observed in the offspring. Stern & Bronner (1970) found that the larger the litter size of guinea pigs, the greater the percentage of time spent by the mother nursing. There was no relationship, however, between the litter size and any other aspect of maternal behaviour. This shows the importance of not assuming that maternal behaviour is governed by a unitary drive; an issue which is discussed later. Rat studies indicate that deteriorating care is associated with increased litter size (Seitz 1954, 1958). Leigh & Hofer (1973) found that, with a litter size of only one pup, rat mothers spent more time adopting a nursing posture, and showed more licking of the pup than control mothers with a large litter. Padmanabhan & Singh (1980) suggested that accelerated development in rat pups from smaller litters, compared to larger litters, was due to differences in the mother-offspring or the offspring-offspring social interactions. Other rat studies indicate that mothers of smaller litters spend more time in the nest with the litter and are generally more maternal (Grota 1973; Grota & Ader 1969). Grosvenor (1956) and Ota & Yokoyama (1967) noticed that rat mothers of smaller litters showed decreased ingestion of food in comparison with mothers of larger litters. This is presumably because the total nutritional requirement of the offspring would be less in small litters.

Kumaresan et al. (1967) found that rat mothers of smaller litters produced less milk than mothers of larger litters, but nevertheless there was more milk per pup. This could explain the faster growth of pups from small litters.

Eisen et al. (1977) also observed that when laboratory mouse mothers were nursing smaller litters, the mothers' food intake was lower, their food conversion efficiency was lower during the first four days and they consumed less during the first 12 days. They also had smaller mammary glands. Perhaps these nutritionally linked facts are not surprising, but Priestnall (1970, 1972) provided some additional behavioural information on laboratory mice. Mothers of smaller litters spent more time in the nest, spent more time nursing, spent more time licking the pups, spent less time eating and drinking outside the nest, self-groomed more and nest-built more. Such mothers gave far more maternal attention than did mothers of larger litters.

Researches on the Mongolian gerbil tend to confirm that certain aspects of maternal care deteriorate with larger litter sizes; for example, time in nest and body contact with pups (Elwood & Broom 1978) and sniffing and licking the pups (Waring & Perper 1980).

Although work has been carried out investigating the effect of litter size on maternal behaviour, the father's behaviour could also be affected. Elwood & Broom (1978) found that in gerbils, the father's behaviour was affected by litter size. When in the presence of a smaller litter, the father spent less time sniffing pups and increased time nest building; two effects which were also displayed by mothers with smaller litters. Fathers with smaller litters also had decreased pup body contact, sniffed and licked pups less, but gnawed the cage bars more often than fathers of larger litters.

In analysing parental and offspring interactions, it is important that a distinction is made between the amount of time a parent is involved in a particular activity and the amount of time a single pup is on the receiving end of that activity. This will obviously be different for a subject pup, depending on the size of the litter; pups in larger litters tending to receive a smaller share of the parental care.

These studies show that the litter size effect is one worthy of further research, especially since the size of the litter may influence parental behaviour, which in turn may affect the physical and behavioural development of the offspring. For this reason I chose to use the litter size variable and investigate its effect on mouse pup development.

2. The Mother's Previous Experience.

Most female mice in the wild will give birth to and rear several litters. Would the behaviour of a primiparous mother (a mother rearing her first litter) be any different from that of a multiparous mother (a mother rearing subsequent litters)? If so, what difference would this make to the pups? I was particularly interested in the effect of maternal experience on the pups' behaviour. Spencer-Booth (1970) identified a number of factors which are likely to influence the way a mother reacts to her offspring. They include the stimuli received from the young, the age of the young, the mother's endocrinal state, the mother's previous experience of young, and the parity of the mother. Richards (1967) suggested that the mother's previous experience could influence later maternal behaviour, either through endocrinal changes during the mother's previous reproductive experiences or through learning effects.

The first question to be addressed is whether the parity of the mother affects her display of maternal behaviour. (In this study, the term 'maternal behaviour' will refer to the behaviour of a mother which serves a caretaking function. Examples of behaviour which fit into this category include nursing, nest-building, retrieving and licking pups.) Some workers have found that the parity of mother rats did not affect the display of maternal behaviour (Grota 1973; Moltz & Robbins 1965). Hartung & Dewsbury (1979) found that parity had little effect on maternal behaviour in white footed mice (Peromyscus leucopus) and in deer mice (Peromyscus maniculatus bairdi). Maternal retrieval of gerbil pups was unaffected by parity (Waring & Perper 1979). It is generally agreed that primiparous rodent females can function as efficient mothers (Richards 1967). Nevertheless, Leblond (1940) found that multiparous lactating mice were more likely to retrieve older pups than were primiparous females. Although mild interest in mouse pups was shown by nulliparous (reproductively naive) females (Brown 1953), lactating females showed far more interest. Previous experience of young pups was seen to be an important factor affecting the display of maternal care in female mice in studies conducted by Noirot (1964a,b, 1972a). Noirot (1964a) found that a higher proportion of female mice showed retrieving, licking and nest-building, and adopted a nursing posture when primiparous compared to when multiparous. Prior reproductive and caretaking experience has been found, therefore, to both accentuate and diminish the display of certain aspects of maternal behaviour.

Several investigations using rats support the view that multiparous females show more maternal behaviour than nulliparous or primiparous females (Bruce 1961; Carlier & Noirot 1965; Seitz 1954,1958; Wiesner & Sheard 1933). A stronger claim seems to be justified for invoking an

endocrine mechanism for this association between parity and maternal experience in rats (Rosenblatt 1969) than in mice (Leblond 1940; Lisk 1971; Richards 1967). Noirot (1964a) suggested that the primary determinant of maternal behaviour in the mouse was the stimulus characteristics of the pups, with hormonal factors serving only to counteract the inhibition of maternal behaviour which was caused by prolonged exposure to the pups and by the pups' increasing age. Gandelman, Zarrow & Denenberg (1970) found that the retrieval performance of a lactating mouse was more pronounced than that of a virgin, when tests were conducted in a novel environment away from the home cage. Hormonal theories were provided to explain this result.

Care must be exercised when comparing the effect of parity on maternal behaviour. It is conceivable that a particular treatment may increase the display of certain aspects of maternal behaviour and decrease the display of others. It would consequently be dangerous to assume that maternal behaviour is governed by a unitary drive (Elwood 1979a; Elwood & Broom 1978; Hinde 1959, 1970). Nevertheless there is evidence to suggest that aspects of maternal behaviour are influenced by the parity of the mother.

The next question which arises is whether changed maternal behaviour will affect the development of the offspring. Epstein & Goldstein (1977) maintained that the behaviour of a pup at weaning was determined by a combination of genetic constitution, intra-uterine experience and post-natal maternal care. Experimental evidence is available to suggest that variations in maternal behaviour are reflected in resulting offspring behaviour. The effect of different maternal environments on the behaviour of mouse pups was demonstrated by Reading (1966). Pups were reared from birth to weaning by foster mothers of either their own or another strain. The two strains used were

BALB/c and C57BL/6. The growth and behaviour of the pups raised by alien foster mothers tended to resemble that of the alien strain and were significantly different from the growth and behaviour of comparable pups raised by foster mothers of their own strain. This is perhaps not a surprising result since the early environment of the mouse pup is dominated by the mother (Richards 1967). It would be most surprising if her behaviour did not, in some way, affect the behavioural development of the offspring.

Maternal experience, due to the parity of the mother, therefore, seems to be a factor which could have a far reaching effect on pup development. For this reason I chose this as the second of the three factors to be used in this project.

3. The Presence of the Father

Once copulatory behaviour is complete, does the father play any continued role in relation to his offspring? There is much evidence to suggest that, in rodents, he does. (In this study the term 'paternal behaviour' will refer to the caretaking behaviour directed towards the pups by the father. This may include forms of behaviour which are traditionally thought of as maternal behaviour such as licking pups, retrieving pups, accompanying pups in the nest and nest-building.)

There is good reason for considering the father as a source of influence in pup development. Porter et al. (1980), working with the precocial spiny mouse (Acomys cahirinus), pointed out that since a postpartum oestrus occurs, it is likely that the father would at least be present until just after parturition in a wild situation. Laboratory evidence is provided to suggest that the rodent father's

role continues throughout the pre-weaning period. Throughout the order of rodents, paternal behaviour is a common phenomenon. It has been observed in some form in the levante vole (Microtus guenthen) (Yardini-Yaron 1952), in the California vole (Microtus californicus) (Hatfield 1935), in the prairie vole (Microtus ochrogaster) (Thomas & Birney 1979), in the Eastern harvest mouse (Reithrodontomys humulis) (Layne 1959), in the Northern grasshopper mouse (Onychomys leucogaster) (Ruffer 1965), and in the golden hamster (Marques & Valenstein 1976; Rowell 1961).

Elwood (1975a,b) investigated paternal behaviour in gerbils. He found that during the development of the litter, the father spent more time in the nest than the mother. The father also built nests and licked and sniffed the pups; although to a lesser extent than the mother. Nest building was performed most often when the mother was off the nest and when the young were exposed. Waring & Perper (1979, 1980) and McManus (1971) also observed the performance of caretaking duties in gerbil fathers.

Animals in the genus Peromyscus and in associated genera have shown paternal behaviour. Horner (1947) observed fathers retrieving young, licking the pups' ano-genital regions and standing over the young in a protective manner. Fathers were seen in the same nest as the mother and pups at 24 hours postpartum, nest-building, licking the young, carrying the young in the mouth, moving pups when the nest was disturbed and washing pups. Even if the father's access to the pups was restricted by the mother during the early part of their development, he still showed considerable care for the pups when they were older (Spencer-Booth 1970). Blair (1941) and Hatton & Meyer (1973) observed as much caretaking behaviour in the father as in the mother. In the

absence of the mother, the father retrieved pups, licked them and stood over them. Although McCarty & Southwick (1977a,b) recorded the mother spending more time in the nest area and showing more caretaking behaviour than the father, the active paternal role of the male was still stressed. Hartung & Dewsbury (1979) claimed that sex differences in parental behaviour were infrequent, and that licking pups, nest construction and retrieval of pups were all performed by the father. It was found by Dudley (1974b) that fathers spent more time in the nest with their pups when either the mother had left the nest or had been removed from the cage. It was also noted that both fathers and mothers cared for the pups equally readily.

Although Barnett (1963) suggested that the male plays no role in the care of the young in the black rat (Rattus rattus) and in the laboratory rat, paternal behaviour has been observed in the laboratory rat (Rosenblatt 1967) and in wild caught Rattus fuscipes (Horner & Taylor 1969).

The laboratory mouse is no exception. Leblond (1940) and Noirot (1964b, 1969b) found that male mice responded in the same way to young pups as virgin, pregnant and lactating female mice. All of these groups exhibited retrieval, licking and nest-building. Little difference existed between males and females in pup retrieval tests. Priestnall & Young (1978) found that male mice displayed as much caretaking behaviour as females. Males were more frequently inside the nest than females.

Elwood (1983) reviewed researches providing information on the time spent in the nest by laboratory mouse fathers and males of other rodent species. He concluded that the adult male spends increasingly

more time in the nest during the first part of the pre-weaning period and spends more time in the nest than the female during the second part of the pre-weaning period.

One could question at this stage whether such behaviour exhibited by the rodent father is typical of that existing in a wild situation. Hartung & Dewsbury (1979) discussed whether it is artificial behaviour resulting from monogamous breeding systems. Elwood (1975b) suggested that it may be widespread amongst mammals and could easily take place in field conditions. Some valuable information was provided by Brown (1953) who studied mice descended from wild animals and bred in a colony. Males were occasionally seen carrying young, but little other interest was shown in them. There are a number of possible factors which could cause paternal caretaking behaviour to be displayed. Lehrman (1961) and Richards (1967) have discussed the possibility of it being controlled by the endocrine system. Such evidence is provided by Leblond (1938) and Riddle et al. (1942), but there is alternative evidence to indicate that experiential effects such as proximity to young may be involved (Leblond 1940; Rosenblatt 1967). As with the mother's caretaking behaviour, a combination of these factors may be at work. Certainly the presence of other adults does seem to affect the extent to which such paternal behaviour is displayed. When adult male mice were housed in communal nests, it was the virgin females which cared for the pups, but when the males were housed alone with the pups, a full range of caretaking behaviour was displayed (Gandelman, Pashke, Zarrow & Denenberg 1970; Lown 1980).

The Father's Effect on the Offspring.

Mugford & Nowell (1972) suggested that there were several ways in which the father could affect the environment of the developing pups. Possible influences include the following:

1. A direct effect; for example, through licking and cleaning.
2. Maintaining body temperature, by being present with the litter in its nest.
3. Non-specific tactile stimulation, again by remaining in contact with the offspring.
4. Interference with maternal care.
5. Providing objects of learning, mimicry or imprinting.
6. Pheromonal influence.

It is possible that the father's participation in the rearing of the litter would be reflected in the offspring's development. Crook et al. (1976) postulated that the father was acceptable to the mother and would stay through gestation and rearing only if his presence significantly enhanced the survival of the offspring.

Elwood & Broom's (1978) gerbil study revealed that pups reared in the presence of the father experienced earlier eye opening and were behaviourally more advanced. Under these conditions the pups spent less time in the nest, spent less time without an adult, walked more, reared more, climbed more but there was no body weight difference or difference in the mortality of the offspring. The major contribution of the father was considered to be that of maintenance of the pups' body temperatures. Waring & Perper (1980), also working on gerbils, agreed that an important role of the father was a thermal one so that most energy could be directed towards growth and development of the pups. Although Foster (1971) found that gerbils raised without the adult

male were heavier than those raised with the male, this conclusion was not supported by the same researcher's subsequent findings.

McCarty & Southwick (1977a) suggested that the male parent had an important influence on the behavioural development of the Southern grasshopper mouse (Onychomys torridus). The presence of the adult male during the pre-weaning period caused an increase in open field activity of female offspring, an increase in male offspring aggression and more efficient predatory behaviour in both sexes; compared to offspring reared without the male. California mouse (Peromyscus californicus) studies have revealed that the father's presence is associated with maintenance of the offspring's body temperature and faster pup development, including the appearance of body hair and weight gain (Dudley 1974a).

In the laboratory mouse, Okon (1971) found that pups reared in the presence of an adult male produced fewer ultrasounds and King & Connon (1955) found the presence of the male facilitated the survival of prematurely weaned mice. Paternal presence appeared to be associated with the development of mating preference (Mugford & Nowell 1972; Yanai & McClearn 1973), with faster sexual and physiological development and with more rapid weight gain in female mice (Cowley & Wise 1972; Fullerton & Cowley 1971). Evidence is available to suggest that this is an odour-induced response. Male odours appear to accelerate sexual maturity in female mice, including the age at which the vagina opened, the timing of the first oestrous cycle and the timing of the first mating (Vandenbergh 1967, 1969). Conner (1972) and Fullerton & Cowley (1971) found that exposing developing female mice to adult males, caused the females to attain their first oestrus earlier and to come into oestrus more readily than control females. Drickamer & Murphy (1978) and Kennedy &

Brown (1970) also found that accelerated sexual maturation in female mice was associated with exposure to adult male odours. In comparison with this, maternal presence and maternal odours seem to have an inhibitory effect on sexual development (Bronson 1968; Bruce 1970; Drickamer 1974). Fullerton & Cowley (1971) also found that female mice reared in the presence of adult males, experienced accelerated weight gain, earlier opening of eyes and ears, earlier eruption of lower incisors, earlier unfolding of pinnae, earlier attainment of full fur and earlier opening of the vagina; when compared to female offspring, reared either in the presence of adult females or in the presence of just the mother. The presence of the adult males was also associated with greater open field activity scores. It was suggested that parental odours were responsible for these effects on pup development and behaviour.

Maternal Mediation of the Father's Effect.

It is possible that the father may affect the pups' development by influencing the way the mother reacts to the pups. Changed maternal behaviour in the presence of the father has been noted in a number of rodent studies

Ahroon & Fidura (1976) found that the presence of the male after parturition was associated with the death of offspring and increased pup mortality in gerbils. This result has been questioned, however (Elwood 1983; Gerling & Yahr 1979; Klippel 1979), and now it is considered that factors other than paternal presence were likely to have been responsible for the pup mortality. Elwood & Broom (1978) found that with the father present, gerbil mothers spent less time nest-building, sniffing pups and gnawing the cage bars. It was suggested that the father reduced the work load and, in turn, the stress of the mother. These same

workers also suggested that the father affected the stimuli received by the mother from the litter, which in turn affected her maternal behaviour. This changed maternal behaviour could then have been responsible for changes noted in the pups when the father was present. Waring & Perper (1980) found that a relationship existed between the nest attendance of the mother and father, so that the pups were seldom left unattended.

Dudley (1974a) suggested that both a direct effect and a maternal mediation effect were operating in the California mouse. In this study, mothers spent less time in the nest and more time ingesting food when the father was present.

Richards (1966a) suggested that altered maternal behaviour, in response to changes in the stimulus properties of the young, could mediate the effect of handling of rodent pups. It is also possible that a maternal mediation effect could provide the route of influence from the father to the pups (Mugford & Nowell 1972).

Some studies, however, have shown aspects of maternal behaviour which have not been affected by the father's presence. Maternal pup retrieval in the gerbil was not significantly affected by the father's presence (Waring & Perper 1979). Priestnall & Young (1978) found that the father had very little influence on the behaviour of the mother and no effect on the physical development of the litter. Later in this study, the possibility of the existence of a maternally mediated paternal influence on mouse pup development is investigated.

Since the father does play an active caretaking role and evidence is available to suggest that pup development is influenced by the

presence of the father, I have chosen paternal presence as the third social factor to be used in this project. Three social factors, therefore, provide the basis for this research project. They are :

1. Litter size.
2. Maternal experience.
3. Paternal presence.

Whereas it is common for researchers to manipulate the environment of developing rodents in order to test their later behaviour (for example, Broadhurst & Levine 1963; Denenberg & Karas 1960; Denenberg & Zarrow 1971; Dubos et al. 1968; Du Preez 1964; Harper 1968; Levine & Otis 1958), this work concentrates on more immediate effects of environmental experiences during development. At no time is the behaviour of any animal studied after day 30; that is, when the animal is 30 days old.

4. Interactions Between the Three Variables.

It is, of course, possible to investigate the effect of each of the three social factors separately, in the laboratory. In a wild situation, however, all three factors would operate together. What would be the effect of interactions between these environmental variables? This seemed to constitute an important question and consequently the main experimental design, described in Chapter 3, was devised with the examination of interactions in mind.

5. Development of the Research Project.

Now that the specific research problems have been defined, it will be possible to look ahead to the rest of the study.

The remainder of this chapter consists of other introductory material on the laboratory mouse and its development period.

Chapter 2 describes my early experimental work. This early work enabled sufficient familiarity to be gained with the behaviour of the research subject to decide upon particular sampling and recording methods.

Chapter 3 outlines the method adopted for the main experiment, which investigated the effect of the three social factors.

Chapter 4 includes the results from the main experiment, with comments on age trends in the performance of the offspring activities which were being monitored.

Chapter 5 is a discussion chapter on the results of the main experiment.

Chapter 6 describes two further investigations into the mechanisms of the paternal presence effect. The purpose of these experiments was to determine whether the father influenced the offspring directly or through the mediation of the mother. The first investigation is called the Split Litter experiment. It involved the division of a litter of nine pups into three groups, exposing each group to a different parental influence. For part of each day, one group was exposed to the

father, another was exposed to the mother and the third was without either parent. The second investigation, called the Split Cage experiment, involved a cage which was divided into two sections. The cage enabled pups to have access to either parent without the two parents having contact with each other.

Chapter 7 provides a general discussion, where the research problems identified in Chapter 1 are examined in the light of the results.

6. Choice of Subject Species.

The laboratory mouse was chosen as the subject species in this series of investigations for several reasons.

1. In a project previous to this one I used the same species as the subject and consequently became familiar with its housing and maintenance requirements. In effect, I had gained several years of experience with this animal and felt confident that this familiarity would provide a sound foundation for more advanced work.

2. The project has involved many hours being spent with the subjects. For a researcher to tolerate and even enjoy this experience, the animals must be personally acceptable to him. The laboratory mouse has satisfied this condition for me since I have found these animals fascinating, interesting and, on many occasions, most amusing.

3. The mouse has a relatively short pre-weaning period, lasting approximately three weeks. During this period, very rapid development takes place. The red, hairless pups, which are restricted to their

nest at the onset of this period, become independent juveniles, exhibiting a wide range of behaviour patterns, at its close. This characteristic allows much data to be collected in a short period of time.

4. Laboratory mice are inexpensive and easy to maintain in large numbers, thus enabling a large sample size to be readily obtained for experiments.

5. The small amount of space taken up by the animals also provides the advantage of modest surface area requirements for cages and other apparatus used for housing the animals.

6. The temperament of this animal makes it very suitable for being housed in laboratory conditions. In the presence of humans, members of this species will breed and rear their young without difficulty.

7. Already there is a substantial body of information on laboratory mice. Tuffery (1967) suggested that this species represented 40 to 80 per cent of all laboratory animals. Each year the research findings from numerous behavioural investigations are integrated into this available knowledge, thus enabling personal findings to be compared and contrasted with others' work. Studies have also been conducted on many related species of rodents; for example, the laboratory rat, the California mouse, and the Mongolian gerbil; and this again provides a context to examine one's own results.

7. Natural History of the Mouse.

An examination of the wild counterpart of the laboratory mouse reveals that it is a very successful animal, living often as a commensal of man. There are few countries in the world which do not support populations of the house mouse. In fact, except for tropical Africa, it lives in a commensal state with man over virtually all of the temperate and tropical land masses of the world (Berry 1970). In the United Kingdom during the summer, Matthews (1968) claimed that it represents the third most common small mammal. Populations of these animals live in fields, hedgerows, woods, farms, houses, warehouses, factories and in many other types of buildings. The high reproductive potential of these animals causes astronomical population sizes to be reached in short periods of time.

Rowe (1973) suggested that it probably has a wider distribution than any other mammal apart from man, inhabiting temperate, tropical and steppe regions. He also pointed out that it could live in a wide variety of different habitats, including cold stores, rice and sugar-cane fields, salt marshes and coal mines.

8. Characteristics of the Developmental Period.

Familiarity with the post-natal developmental period of the laboratory mouse is necessary in order to appreciate the importance of the social variables which the developing pups were subjected to in this project.

Females are polyoestrous and, in common with many other rodents, possess a high reproductive potential. With a gestation period of

only 21 days and with frequent pregnancies resulting from the post-partum oestrus, females can give birth to many litters each year. Under laboratory conditions, most strains of mice will produce litters with at least seven pups in them. The rapidity with which litters follow one another means that one group of pups might only be three weeks old when the attention of their parents is directed away from them and towards a newly born litter. Pups can easily survive at this age without being dependent upon their parents, indicating that their development is a very rapid one.

Williams & Scott (1953) have investigated the development of social behaviour in the mouse. They carried out ten minutes of observation each day from birth until the pups were 30 days old. Four natural periods were observed beginning at 0, 4, 12 and 25 days. They were described as follows :

Period 1. Neonatal Period. During this period the pups were naked and largely helpless. They showed mostly ingestive and contactual behaviour, and did not react to sound or sight. There was no indication of learning capacity, although this was not formally tested by the authors.

Period 2. Transition Period. This involved the rapid development of sensory, motor and psychological capacities which provided the basis for future changes in social and grooming behaviour. There was an increase in investigative behaviour during this period. Some reaction to sound was noted early on in the period and there was evidence of a startle response near its close.

Period 3. Socialization Period. At the beginning of this period all of the sensory organs were functional. During it, there was rapid motor development and the appearance of all the characteristic social behaviour patterns typical of adult behaviour. The predominant activities were investigative and grooming behaviour. There was great sensitivity to frightening stimuli, with nursing disappearing at the end of this period.

Period 4. Juvenile Period. Fighting appeared during this period, and at its close, adult sexual behaviour had developed.

In contrast with this descriptive and mainly non-manipulative study, Fox (1965) monitored the development of the mouse by subjecting pups to a barrage of reflexological tests each day. The results from these tests were used to indicate the extent to which neurological development had taken place. Once again the period of development was divided into separate stages. This time five stages emerged. They were as follows :

Period 1. Perinatal Period (0 to 3 days.)
At this stage a bilateral stimulation of the face region caused the animal to crawl forwards. When the animal was turned on its side, it could right itself, and when it was placed on a 45° slope with its head pointing downwards it would turn through 180° and crawl upwards. If the dorsum of the foot was rested on the edge of an object, the foot would be raised and placed on the object. These four reflexes were weak at this stage.

Period 2. Neonatal Period (3 to 9 days).
By now the above reflexes were strong. A pivoting and circling mode of locomotion was observed.

Period 3. Postnatal (transition) Period (9 to 15 days).
At this stage these earlier reflexes were disappearing whilst the organs of special sense were becoming functional. The body vibration called hyperkinesis disappeared at the end of this period and over-generalized sensory responses (in which pups responded to tactile and auditory stimuli with gross body movements) were observed.

Period 4. Postnatal (infantile) or Pre-juvenile Period (15 to 26 days).
Here there was a refinement of the pups' sensory and motor abilities. The over-generalized responses to sensory stimuli were now disappearing.

Period 5. Juvenile Period (26 days until sexually mature).
A further refinement of sensory and motor skills took place so that the subjects could manipulate objects in an adult manner.

These two studies complement each other and strongly suggest a link between the rapid behavioural development on one hand and the continually maturing nervous system on the other. Fuller & Wimer (1968) have provided a summary of pup activities based on the above two sets of researchers' findings. Their summary is as follows :

On the day of birth the pups could crawl sideways.
On days 3 to 4 they could pivot round using their forepaws.
On days 6 to 7 they could stand and walk.
On day 9 they could run unsteadily.
On day 15 they could move as an adult.
Even up to day 20 they stayed close together.

During the third week they were very fast and active, taking long leaps around their cage. At this stage they were appropriately called 'popcorn' mice.

Wahlsten (1974) also established a time scale for laboratory mouse postnatal development. For this he employed reflexological tests, measures of body weight, myelination of 80 fibre tracts and the thickness of the external granular layer of the cerebellum.

Another important aspect of the early development of laboratory mice is that of temperature regulation. Lagerspetz (1962) and Okon (1970a) have examined the postnatal development of homoiothermy in the laboratory mouse. Okon (1970a) found that albino mouse pups develop homoiothermy during the first 19 days after birth. Three distinct stages of development were identified. These were as follows :

1. (days 1 - 6) The pups were almost completely poikilothermic at low ambient temperatures during this stage.
2. (days 7 - 14) This was the transitional stage when the pups developed homoiothermy at temperatures around 22^{o}C.
3. (days 15 - 19) The final stage was characterized by the development of full homoiothermy even at low temperatures.

Linked to the development of temperature regulation is the production of cold-induced ultrasounds. Okon (1970a) found that the ultrasounds were weak during days 1 to 6, but were much stronger from days 7 to 13. Then from day 14 onwards, the pups progressively stopped producing ultrasounds. Noirot (1972b) found that although cold-induced ultrasounds were infrequent and quiet during the first few days after birth, they increased in frequency and intensity with the development of homoiothermy.

Ultrasounds are also triggered by the reception of tactile stimulation. It is reported that these ultrasounds follow a different pattern to those induced by cold. They begin with very high intensity pulses in very young pups, but gradually decline with increasing age of the pups (Noirot 1972b; Okon 1970b). Noirot (1966) found that the ultrasounds suddenly rose when the pups were 4 days old and their ears had opened, and that the ultrasounds had practically ceased by 14 days of age, when the pups eyes had opened.

The Parental Response to the Offspring.

During the time the pups were developing, the parents were involved in caretaking behaviour. A hollow nest would have been constructed prior to parturition and right from birth the young mice would receive much parental attention, including nursing, licking and body contact. Such attention is vitally necessary because the altricial laboratory mouse pup, unlike some precocial rodent offspring such as the spiny mouse (Acomys cahirinus) (Porter et al. 1980), is totally dependent on its parents for nutrition, protection and temperature maintenance. Noirot (1972b) found that one of the parental attractants was the cold-induced ultrasounds produced by the pups. These ultrasounds induced retrieving and nest-building.

Noirot (1969b) showed a range of responses adults carry out in the presence of young mice. One hundred and fifteen naive adults were given a standard 5 minute test with a 1 to 2 day old pup. The adult subjects comprised both males and females, yet they all retrieved the pup. One hundred and thirteen licked the pup, 75 exhibited nest-building behaviour and 80 displayed a nursing posture.

It would appear that parental caretaking responses diminish in intensity as the pup increases in age. Such responses seem to depend mainly upon external stimuli arising from the pups rather than on hormonal changes (Leblond 1940; Noirot 1964c). Meier & Schutzman (1968) claimed that the mother is sensitive to the changing properties of the rodent pup.

Throughout the early postnatal period of the pups, both parents and offspring are changing in their behaviour to one another, in a developing pattern of reciprocal interactions (Bronfenbrenner 1968; Rosenblatt 1965). One such interaction is that of the offspring feeding from the mother.

Certain characteristics of the developing offspring and their parents are particularly relevant to this project and are listed below.

1. Newly born pups, although far from being independent initially, develop rapidly so that by three weeks of age they do not depend upon their parents for survival.
2. The behaviour of the pups, during this developmental period, changes rapidly. It is a relatively easy matter to monitor relationships between the pups and the parents; and the pups' display of common activities such as grooming, exploratory behaviour, locomotor behaviour and feeding.
3. The pups of one litter tend to stay together during most of this developmental period. Whether this mutual affinity is caused by physical limitations or by social preferences, a dominant feature in the developing pup's environment will be its littermates. The influence of the litter on the constituent pups is, consequently, worthy of study.

4. The attention given to the young animals by the mother is considerable and she consequently represents a powerful source of stimulation during the period of development. What effect the mother's experience has on the development of the offspring, constitutes an important question.

5. Other adults share the caretaking role with the mother. What exactly the father's role is and the effect this role has on the pups' development, are lines of enquiry which can be pursued.

6. Through being in close proximity to one another, a complex set of relationships exist between the pups in the litter, the mother, the father and other adults which are present. When considering the effect one animal has on another, both direct effects and indirect effects must be considered. For example; a mother may be affecting her offspring directly or, alternatively, she may be affecting the father who, in exhibiting changed behaviour, affects the offspring. I shall term such indirect effects, 'mediation effects'.

Chapter Two

PRELIMINARY EXPERIMENTAL WORK

CHAPTER 2

PRELIMINARY EXPERIMENTAL WORK

1. Objectives

The early experimental work of this project was intended to fulfil three main objectives. These were :

(i) to develop some familiarity with the biology of the laboratory mouse in order to provide a foundation of knowledge on which future research lines could be based.

(ii) to investigate the nature of pup development during the pre-weaning period.

(iii) to develop sound experimental techniques, especially for recording behaviour.

This experimental work was an essential preliminary to the subsequent main experiment. Before a specific experimental design could be contemplated, a number of questions needed to be answered. The more pertinent questions are outlined below.

(i) Biology of the Laboratory Mouse

1. Which strain of mouse would be most suitable to provide subjects?
2. What are the basic, physiological requirements of the subjects? (I was anxious that no physiological limiting factor, such as a nutritional deficiency, should be reflected in the results of, what was essentially, a piece of behavioural research.)
3. To what extent could the members of a family be manipulated? Here, the effects of the removal of the father and of the members

of a litter were considered to be important. Cannibalism in rodents is a well-known phenomenon (Elwood 1980; Gandelman & Vom Saal 1975; Rowell 1961) yet its occurrence would be most unwelcome in these researches.

4. How many pups are usually born in a litter? This information was required in order to determine the litter size variable levels.
5. Which cage system would prove most suitable for meeting the basic requirements of the subjects whilst also providing adequate observation opportunities?

(ii) Nature of Pup Development.

1. At what age could recording start and when should it be concluded?
2. Which behavioural patterns would be worth recording? A pilot study of pup development would be necessary in order to ascertain how to monitor the effects of the variables being tested.

(iii) Experimental Techniques.

1. Which approach would prove most suitable for adoption in this project? There are several logical approaches to take:

(a) The non-manipulative and descriptive approach which was used by Williams and Scott (1953) whilst investigating the social behaviour of the laboratory mouse. With this approach the emphasis is on observation of as natural behaviour as is possible under laboratory conditions. The subject animals are handled as little as possible and are consequently unlikely to be greatly affected by this research approach.

(b) The manipulative approach which can involve a series of reflexological tests and which was used by Fox (1965) whilst studying

mice. When adopting this approach, it is accepted that the developing pups will be handled excessively. Pups are removed from their cages in order to administer the tests. Interest is shown in the responses of the pups to these specific tests rather than to the display of their natural behaviour. The subjects will undoubtedly be affected behaviourally due to their treatment. Wimer and Fuller (1968) have reviewed researches linking early post-natal environmental experiences with behavioural changes in mice.

(c) A combination of the two approaches outlined above; where social behaviour and inter-subject behaviour is monitored, but also where some degree of human manipulation is involved in the experimental design.

The exact nature of the approach which is utilized will be determined by the research questions which are to be answered in this study.

2. How should the recording be carried out? In order to treat the data statistically, it should exist in a quantitative numerical form.

3. At what time during the day should behaviour be observed and recorded?

2. Early Experimentation

The work outlined below is described in sufficient detail to show how :

(a) the above questions were answered.

(b) the earlier experimental work was instrumental to the development of later experiments.

(i) Biology of the Laboratory Mouse.

1. Strain of Mouse. Initially, laboratory mice were used which had been obtained for genetic studies. There were two types represented. One was a black variety (++aa) from Phillip Harris Biological Ltd., Oldmixon, Weston-Super-Mare, Avon BS24 9BJ. The other was a yellow variety ($A^{y}a^{t}$) obtained from Griffin Biological Laboratories, 113 Lavender Hill, Tonbridge, Kent.

These proved adequate for initial studies while basic questions about the biology of the laboratory mouse were being answered, but then a single strain of mouse was required specifically for this work. Each strain possesses specific characteristics (LaBarba 1967; LaBarba et al. 1968; Newell 1967; Ralls 1967; Ressler 1963; Smith & Powell 1955) and it became necessary to eliminate these inter-strain differences which would tend to contaminate experimental data. It is claimed that strain differences can account for large differences in behaviour, maturation rates and open field emotionality scores (Denenberg 1963a; Moltz 1971). The random bred CFLP strain was obtained from Carworth Europe, Huntingdon.

The CFLP strain was recommended to me on the grounds that the animals were hardy and disease resistant, were readily available, and that they bred well under laboratory conditions. When this new stock

arrived, animals used for experimentation were kept separate from those animals used solely for breeding. Tuffery (1967) stressed that research work based on the use of young suckling mice necessitated the production of these animals exactly where they would be used. From this point onwards, all parents and pup subjects were produced on site.

In order to ensure that all subjects received a uniform developmental environment, certain pairs of animals were used solely for the production of new experimental subjects. Animals used for experimental purposes were never used again for subsequent experiments or for breeding purposes. Denenberg (1969), Levine (1960,1962a,b), and Harlow & Harlow (1962) are just a few of the workers who have shown the later effect of variable conditions in the early experience of an animal. The relevance of this effect to rodents has been demonstrated experimentally (Denenberg et al. 1966; Hall & Whiteman 1951; Kapland & Hyland 1972; Manosevitz 1970). The uniform developmental environment in my own work included housing conditions, nutritional factors, the amount of handling received and the amount of contact that was possible with other animals. The environmental temperature was maintained throughout at 20°C and the relative humidity was between 50 and 60%. These precautions were taken to help ensure that the future experimental results would not be affected by differences in the parents caused by variations in their early pre and postnatal environments.

Much of the work described in this chapter was carried out on the CFLP subjects. Before the main experiment, which is described in Chapter 3, was begun; another change was made in the strain of mouse. I decided to complement the environmental uniformity with greater genetic uniformity by using an inbred strain of mouse. Fuller & Wimer (1968) described the value of inbred mice to the research worker

who was interested in identifying environmental sources of behavioural differences. They further maintained that genetic variation within an inbred strain could be safely ignored. McClearn et al. (1970) claimed that the main advantage of an inbred strain was the stability of its mean over consecutive experiments; and suggested that mice originating from a common origin but maintained in different laboratories would accumulate genetic differences only very slowly and remain comparable for long periods of time. Tuffery (1959) pointed out that the disadvantage with inbred mice was the small litter size obtained, but that this was outweighed by the advantage of not having genetic differences influence the experimental results. Consequently BALB/c mice were obtained, and all subjects in the experimental work described in Chapter 3 onwards were members of this strain. The laboratory history of this strain stretches back to 1917 when they originated from a dealer's stock in Ohio, U.S.A. These origins are explained in Heston (1945), MacDowell et al. (1927) and Staats (1968). BALB/c mice have been used frequently in behavioural studies (Wimer & Fuller 1966), and some researchers (Ginsburg & Allee 1942) have claimed that they are a relatively non-aggressive strain. It has been reported that they are characterized by a long courtship period (McGill 1962), readily hoarding food (Smith & Powell 1955), food sharing with other members of the same strain (Fredericson & Birnbaum 1954), a high incidence of defaecation in open field test apparatus (Thompson 1953), a fast development of avoidance conditioning (Royce & Covington 1960) and low open field activity scores (Thompson 1953). After comparing two strains of mice, Ressler (1962) claimed that as parents, BALB/c mice handled their own and other strain pups most frequently; and as pups, were handled most frequently by their own and other strain parents. Pups also obtained higher scores in a visual exploration test when reared by BALB/c parents, compared to other strain parents (Ressler 1963).

The original BALB/c mice used in this laboratory were obtained as four trios from Olac Southern, Bicester in July 1975. During the time of their use in this experimentation they have proven most satisfactory in terms of litter production (frequency and size), caretaking behaviour, low pup mortality and low levels of aggressive and antagonistic behaviour. All subjects have been taken from this stock for this research project so that the genetic variation effect could be minimized and emphasis placed on the environmental effects.

A mouse breeding programme operated as follows. When mice were 4 weeks old they were removed from their parents and placed in separate cages in pairs so that breeding could commence. Tuffery (1959) claimed that monogamous pairs have better health records than harem-bred mice. Although strict inbreeding procedures were not adopted, pairs of mice were always closely related. Such cages, containing mating pairs, were placed back in the mouse production zone, or were set aside for experimental observation. Animals which showed abnormal behaviour, such as poor caretaking behaviour, or which produced sub-standard litters (condition or size) were removed from the system. It should be emphasized that only on very few occasions has this been necessary, and there has been no incidence of disease.

2. Physiological Requirements. Early problems were encountered with the BALB/c animals. An Oxoid 41B rodent diet obtained from Oxo Ltd., Southward Bridge Road, London, was used with great success with the CFLP strain mice. Serious physiological symptoms developed, however, when this diet was used with the inbred BALB/c stock. Incidence of pregnancy and litter size was low, pup mortality was high and the condition of the animals was poor. This condition was characterized by an arched back and poor coat condition.

Diet 41B was developed as a complete diet requiring no supplements (Bruce & Parkes 1949); but as Tuffery (1967) acknowledged, different strains of mice have different nutritional requirements. Blackmore & Williams (1956) pointed out that some standard diets are nutritionally inadequate for some mouse strains. Such inadequacies have manifested themselves in reduced breeding performance and lower weight gains (Porter et al. 1963).

I learnt from personal conversation that this detrimental effect was not uncommon in BALB/c mice. The problem was rectified with the introduction of 'Production Mouse Diet' nuts obtained from Labsure Animal Diets, The Christopher Hill Group Ltd., Agrarian House, Castle Street, Poole, Dorset. No nutritional deficiencies have been noted since that time.

3. Manipulation of Family Composition. Because I intended to conduct experiments involving the removal of individual members of a family of mice, it was important to carry out pilot investigations into the effect of manipulating the composition of the family. It was necessary to have information on the degree of manipulation that was possible without causing cannibalism or desertion of the young by the parents.

The gestation period for the laboratory mouse is around 21 days long. Once a female was recognizably pregnant with a swollen posterior abdomen, she was handled and moved from one cage to another. Thirty animals were treated in this way and in all cases a healthy litter was born which was treated normally by the mother. This meant that in setting up cages for the experimental animals, pregnant females could be safely transferred from an original cage to a freshly cleaned cage.

In another 30 situations the father was removed immediately after the litter was born. Removal of the father apparently caused no distress to the mother and she continued to display normal caretaking behaviour towards the litter in each one of the 30 experiments. The litters survived to adulthood in all cases. Removal of members of the litter on day 0 (within 24 hours of being born) elicited some pup-directed antagonistic behaviour towards the remaining pups by the parents. This was not anticipated and when parents showed cannibalistic tendencies the experiment was terminated. The mother was primarily responsible for this behaviour.

The mother showed more cannibalistic behaviour than the father and this is consistent with the findings of Elwood (1980) in gerbils (Meriones unguiculatus) and of Marques & Valenstein (1976) in hamsters (Mesocricetus auratus). Gandelman & Vom Saal (1975) found the opposite was the case in mice, but their experimental circumstances were somewhat different from my own. In a further 20 experiments, members of the litter were removed on day 2 (when the pups were 2 days old) and in only one experiment did any parent show any cannibalistic behaviour. This in fact was the mother, but such a result does not represent a higher proportion than is found under normal circumstances, when the litter is not interfered with.

The above findings enabled a policy to be established regarding the timing of removal of members of the family. Under circumstances where it became necessary to remove the father, this was carried out immediately after the litter was born and prior to the postpartum oestrus. Litter size adjustments were carried out when the pups were two days old.

In order to ascertain whether there was a minimum size litter that would survive to weaning, 10 litters were reduced to two pups and 10 litters were reduced to one pup. Whereas nine out of the two-pup litters showed no pup mortality, only six out of the one-pup litters reached 10 days of age. Such a finding is explicable in terms of a minimum suckling stimulus required for continued lactation. It seems probable that one single pup does not constitute a sufficient stimulus for the mother to continue lactating. That a single pup is not able to elicit a full milk-ejection reflex from the mother and that, consequently, an optimum litter size is required for pup development; is an issue addressed by several authors. Elwood & Broom (1978) commented on this phenomenon and provided supporting evidence for it from their study of litter size in gerbils. Harris (1958) claimed that the suckling stimulus provided by young mammals induced pituitary gland secretions responsible for the maintenance of lactation. Were this stimulus to fall below the necessary threshold, the endocrinal link would be interrupted. Hammond & Marshall (1925) described an experiment where the young were removed from rabbit mothers (Oryctolagus cuniculus). The mammary glands became inactive as a result, but in the same discussion it was pointed out that lactation could be prolonged by keeping the young with the mother. The suckling stimulus, the authors claimed, must not only cause posterior pituitary hormones to be secreted, thus causing milk ejection, but also stimulate the secretion of the anterior pituitary hormones which are responsible for lactation. Selye (1934), in an early study, removed young pups from mother rats. Milk secretion ceased within a few days and shortly afterwards there was atrophy of the milk secreting tubules. This, once again, indicates the necessity of a sufficient suckling stimulus for continued lactation. Bruce (1961) extended the period of lactation for up to 12 months in the rat by continually substituting an older

litter with a much younger one. Here lactation was continued by providing a sufficiently intensive suckling stimulus for a longer period than would have been possible with the original litter. Further information on the milk secretion mechanism is found in Rosenblatt & Lehrman (1963), but it should already be appreciated that care must be exercised in order to avoid using a litter size too small to constitute an effective suckling stimulus for the maintenance of lactation.

Leigh & Hofer (1973) found that pup mortality was increased in very small litters and Kumaresan et al. (1967) indicated a relationship between very small litters and underdevelopment in pups. Both of these findings can be associated with inadequate milk ejection. Because of the better survival record, I decided to use a litter of two pups as the minimum size. This also provided the advantage of investigating inter-pup relationships, even with a small litter size.. With one pup, it is only possible to observe pup-parent interactions.

All of my pilot experiments reported so far, were conducted on primiparous parents. A small number of additional experiments indicated that multiparous parents reacted just as satisfactorily to the above conditions.

With the above investigations completed, it was evident that litter size, maternal experience and paternal presence could constitute workable variables for future research.

4. Expected Litter Size Richards (1967), in referring to common laboratory mouse strains, indicated that although large strain differences do exist, the mean litter size is between six and ten pups.

In order to identify an upper litter size for later experimentation, litter size data were obtained from 51 mated pairs of mice that had each produced three litters. Litter sizes were counted when the pups were two days old. The litter sizes were compared using a one way analysis of variance. Table 2.1 shows the analysis summary and Fig. 2.1 shows the results in histogram form. The analysis of variance test has been extensively utilized in this research project. Chapter 3 contains additional details concerning its use. Even in the litters of primiparous mothers, which were significantly smaller, a sufficient proportion had seven pups in the litter to make it possible to use seven as the upper litter size in the main experiment.

It was decided to use parents with their own offspring throughout. If foster pups are added to reach a specific litter size, additional uncontrolled variables are introduced (Wimer 1962). If foster pups are used at all, it would be preferable to make all pups, foster pups (Ackerman et al. 1977), but in my own work it has not been necessary to use any. Required litter sizes were, therefore, obtained by culling on day 2.

5. Selection of Cage System. There were two basic requirements of the cages used. One was the need to provide a satisfactory environment for the laboratory mice. There would have to be the provision for the dispensing of food and water, and physical facilities for nesting and for movement. If the area was too cramped, a full range of behaviour would possibly not be manifested. The second requirement was that of providing the observer with the opportunity to view the subjects at any time.

Table 2.1

Analysis of Variance of Litter Size Data

With Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
PARITY OF MOTHER	2	30.05	4.32	<0.05
RESIDUAL	150	6.95		

The following abbreviations have been used:

df : degrees of freedom

MS : mean square

F : the F ratio

P : the probability level

Scheffé Test

PARITY OF MOTHER

	1ST LITTER	3RD LITTER	2ND LITTER
Means	4.980	6.176	6.412

Any two means not underlined by the same line are significantly different. Any two means underlined by the same line are not significantly different (Duncan 1955).

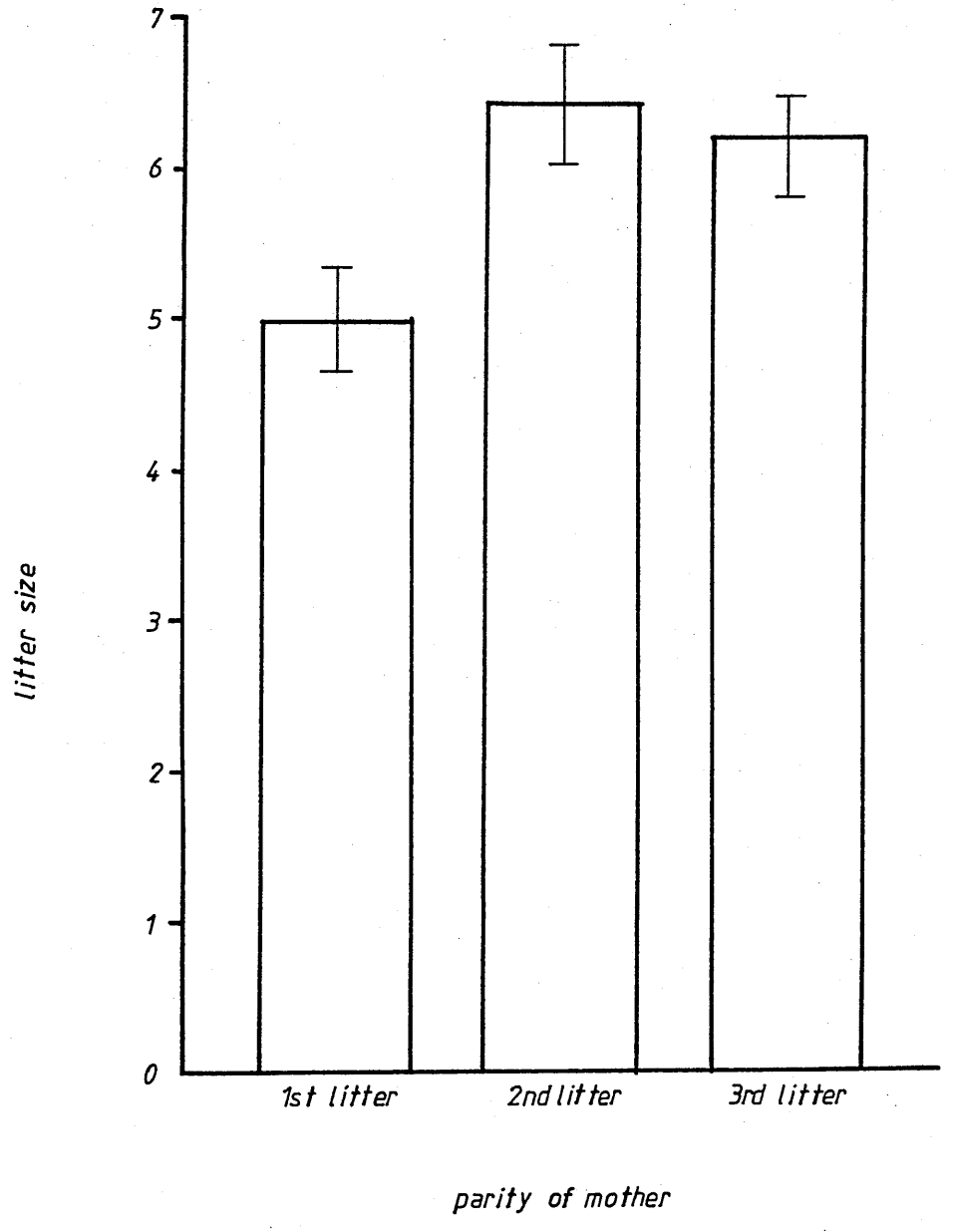


Fig. 2.1 *Litter size means obtained from three different levels of maternal experience.*

Many different cage types were tested including the much recommended Cambridge Mouse Cage from Griffin Biological Laboratories. Although this appeared to satisfy the first requirement, it totally failed to satisfy the second. The top, which incorporated food and water recesses, completely obscured the view of the animals. Most commercial rodent cages seemed appropriate for breeding rodents, but proved inadequate for observation purposes.

Eventually Philip Harris Ltd., 63 Ludgate Hill, Birmingham; were able to obtain a satisfactory cage (Ref. no. B5018). This has base measurements of 300 x 170mm and a height of 100mm. This much greater height and flatter top provided easier visual access to the contents of the cage. Again, environmental uniformity was essential for all subjects and their parents, and so 50 cages of this type were obtained. The cages had opaque polypropylene bases and metal grid tops. The tops housed a water bottle and food (Figs. 2.2 and 2.3).

A 25mm depth of softwood sawdust was provided in each cage. Initially, absorbent paper strips were also provided to act as nesting material. This practice was soon discontinued because its use, by the parents in nest construction, obscured the view of the young litter. The provision of a few small-size wood shavings constituted an improved substitute. Nests were constructed as depressions in the sawdust with the inclusion of wood shavings around the perimeter. These nests appeared to be totally satisfactory to the mice and provided a clear view of the nest contents. It will be appreciated that such a view is absolutely crucial in an investigation of this type.

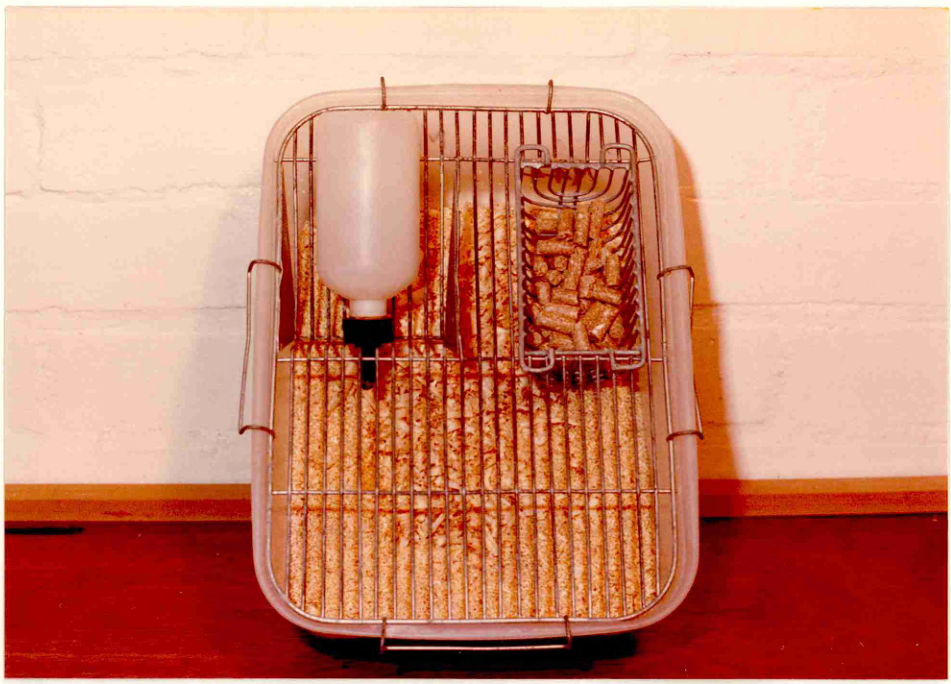


Fig.2.2 View from above, of standard cage

Fig. 2.3 Oblique view of standard cage

(ii) Nature of Pup Development

Williams & Scott (1953) and Fox (1965) have examined very carefully the development of behaviour in the laboratory mouse from birth to adulthood. Their findings are reviewed in Chapter 1. It was not my intention to repeat the work of these researchers, but rather to become familiar with the stages of social development during the first few weeks of life. Such a familiarity enabled decisions to be made regarding the recording of behaviour. The following two sections concern two such decisions.

1. At What Age Could Recording Start and When Should It Be Concluded? Preliminary experiments were conducted which were solely instrumental in establishing guidelines for future research. These are referred to briefly in this section.

It should be apparent that recording of pup behaviour could, theoretically, begin on day 0 (the day of birth) and continue right through to adulthood. Certain limitations, however, were imposed on the length of time for which recording was deemed desirable. Routine observations from birth onwards revealed that, for a large proportion of the time, the parents were in the nest with the litter. Day 16 was the earliest that the pups were observed leaving the nest for extended periods. Up to day 12 they were in the nest for most of the observation sessions.

Four litters and their parents were observed with single instantaneous scans, at randomly selected times during the day to investigate the amount of time pups were visible in the nest due to the nest being unattended by either parent. These observations were made between 0900 hours and 1800 hours, and during the light phase of the day/night cycle. For each observation, I just recorded whether the nest was attended or unattended by the parents.

The results are shown in Table 2.2. Twenty readings were obtained every two days beginning on day 2 (when the pups were two days old). (The results shown in Tables 2.3, 2.4 and 2.5 were obtained in the same way.) For the first few days, nest attendance by the adults was high, and this caused great difficulty to the observer because the view of the pups was interrupted. This view became less interrupted with time, due to decreased parental nest attendance and due to an increase in the size of individual pups. Because of this, I decided to begin recording the social behaviour of the pups on day 10. As will be discussed later, manipulative investigations were also carried out, but these were not limited by the parents obscuring the view of the pups.

As explained in Chapter 1, the brief I had set for myself in this research was to examine the behaviour of the mouse during its pre-weaning period. Weaning takes place at different ages for different rodent species. Svihla (1932) reported that in the white footed mouse (Peromyscus leucopus) it began as early as 22 days, but could occur as late as day 37. In the same study it was found that the California mouse (Peromyscus californicus) was not weaned until around day 44. Novakova (1966) concluded that 30 days was the optimum age for weaning in rat pups. Richards (1967) stated that the age for weaning of laboratory mice was 20 to 25 days. This is referring to common strains of mice and it is acknowledged that there are strain differences.

Continuous daily observations with my own mice revealed that suckling continued until day 28, but that by this age the pups were largely nutritionally independent of their parents. During the 14 to 18 day period a number of behavioural trends accelerated and these indicated the increasing physical independence of the pups from their parents. Eating solid food was first observed on day 18, as shown in

Table 2.2

Mean Percentage of Total Number of Observations in which Nest Was Left Unattended By Parents

(Standard errors are given)

DAY	MEAN SCORES (% OF TOTAL No. OF OBSERVATIONS)
2	11.25 ± 4.25
4	16.25 ± 3.75
6	15.00 ± 6.10
8	18.75 ± 7.20
10	23.75 ± 3.75
12	28.75 ± 6.90
14	21.25 ± 2.40
16	21.25 ± 16.25

Table 2.3

Mean Percentage of Total Number of Observations in which Pups Were Eating Solid Food

(Standard errors are given)

DAY	MEAN SCORES (% OF TOTAL No. OF OBSERVATIONS)
2	0
4	0
6	0
8	0
10	0
12	0
14	0
16	0
18	1.25 ± 1.25
20	2.50 ± 1.45
22	2.50 ± 1.45
24	12.50 ± 4.35
26	16.25 ± 4.75
28	31.25 ± 9.45

Table 2.3. This is consistent with the observations of Williams & Scott (1953).

From day 14 onwards the incidence of exploratory behaviour by the pups increased. It had existed at a low level up to this point; but then, as Table 2.4 shows, there was an increase. This exploratory behaviour consisted of sniffing, lifting and turning the head, and completely rearing up on the hind limbs.

Another interesting developmental trend is that of self-grooming. This experienced a spurt from day 16 onwards as shown in Table 2.5.

Williams & Scott (1953) add that around day 12, simultaneous or synchronous behaviour; which includes suckling, grooming and locomotor behaviour; disappears. This appears to coincide with the time pups begin to leave the nest and represents a step towards independence from other animals. Williams & Scott also note that by day 25 early, but ineffective attempts at copulation can take place. Whilst I did not observe this latter occurence at such an age, it is clear that by day 25 major changes characterized by the pre-weaning period have already taken place. Day 25, therefore, was regarded as a suitable time for the end of the recording period. For the main experimental phase, then, recording was carried out from day 10 to day 25 inclusive.

Throughout this work the expressions 'recording session' and 'recording period' refer respectively to the daily session of observation and recording, and the entire length of time in days for which recording was carried out.

Table 2.4

Mean Percentage of Total Number of Observations in which Pups Were Exhibiting Exploratory Behaviour

(Standard errors are given)

DAY	MEAN SCORES (% OF TOTAL No. OF OBSERVATIONS)
2	0
4	0
6	1.25 ± 1.25
8	1.25 ± 1.25
10	2.50 ± 2.50
12	1.25 ± 1.25
14	3.75 ± 2.40
16	7.50 ± 3.75
18	3.75 ± 2.40
20	8.75 ± 4.37
22	6.25 ± 3.75
24	5.00 ± 2.90
26	11.25 ± 4.25
28	7.50 ± 2.50

Table 2.5

Mean Percentage of Total Number of Observations in which Pups Were Self-Grooming

(Standard errors are given)

DAY	MEAN SCORES (% OF TOTAL No. OF OBSERVATIONS)
2	0
4	0
6	1.25 ± 1.25
8	0
10	2.50 ± 2.50
12	1.25 ± 1.25
14	2.50 ± 1.45
16	15.00 ± 5.40
18	5.00 ± 2.05
20	7.50 ± 3.20
22	12.50 ± 1.60
24	13.75 ± 5.55
26	16.25 ± 4.75
28	11.25 ± 2.40

2. Identifying Pertinent Behavioural Patterns. During these routine pilot studies, certain pup behavioural patterns were very easily noted. I have already referred to suckling, eating solid food, exploration and self-grooming.

There is obvious value in noting which categories of pup behaviour were of interest to other workers. Porter et al. (1980) considered suckling, physical contact, eating solid food, grooming, biting and chasing, in spiny mouse pups (Acomys cahirinus). Elwood & Broom (1978) focussed their attention on the time spent by gerbil pups in the nest, near to the mother, near to the father, away from any adult, suckling, walking, self-grooming, grooming others, scratching, rearing, wrestling, chewing (not the cage bars) and climbing.

Bolles & Wood (1964) used the following categories during their study of the ontogeny of behaviour in the laboratory rat: sleep and rest, consummatory behaviour, grooming, locomotion, exploratory behaviour, burrowing, fighting and playing.

The behavioural categories eventually adopted in this study evolved by trial and error from earlier, less satisfactory lists. Twelve categories were ultimately considered important and worth recording. These were; presence of the pup in the nest, proximity to littermates, proximity to the mother, proximity to the father, locomotor behaviour, three categories of exploratory behaviour (sniffing, head-lifting and rearing), self-grooming, suckling, eating solid food and drinking water. Some of these categories are shown in Figs. 2.4 to 2.9.

A developmental milestone is reached when pups leave the nest for the first time. A certain level of physical maturation and motor

co-ordination is necessary before pups are able to leave the nest. Proximity to other animals was determined by counting the times when the pup's head was within 1cm of the animal. It is to be expected that littermate proximity scores would be high at an early stage when pups are spending the majority of their time together in the nest. When pups are able to position themselves anywhere in the cage, this measure would provide additional information on their tendency to remain near each other. Mother proximity and father proximity scores would be determined by the parents initially, but later these scores would reflect combined interests of both offspring and parents (Fig 2.4).

Locomotion is an umbrella term including walking, running, climbing and digging. The type of locomotor behaviour displayed, would depend upon the motor development of the subjects. Information on activity rates would also be provided by monitoring the amount of locomotor behaviour exhibited by the pups (Fig. 2.5).

The three types of exploratory behaviour would provide a measure of motor development, and also of the tendency of the subjects to explore their environment. It should be appreciated that the three activities; sniffing, head-lifting and rearing; are progressive in ontogeny. Sniffing occurs first and is associated with a characteristic head position. Head-lifting incorporates sniffing and appears later in the development of the subjects. In the same way rearing, which requires much more effort, incorporates the other two exploratory activities (Fig. 2.6). Because of this, 'sniffing' refers only to sniffing; 'head-lifting' refers to head-lifting and sniffing; and 'rearing' refers to rearing,head-lifting and sniffing. Animals which have developed the ability to rear, will still display head-lifting and sniffing as separate activities.

Fig. 2.4 Young pups in nest with parents

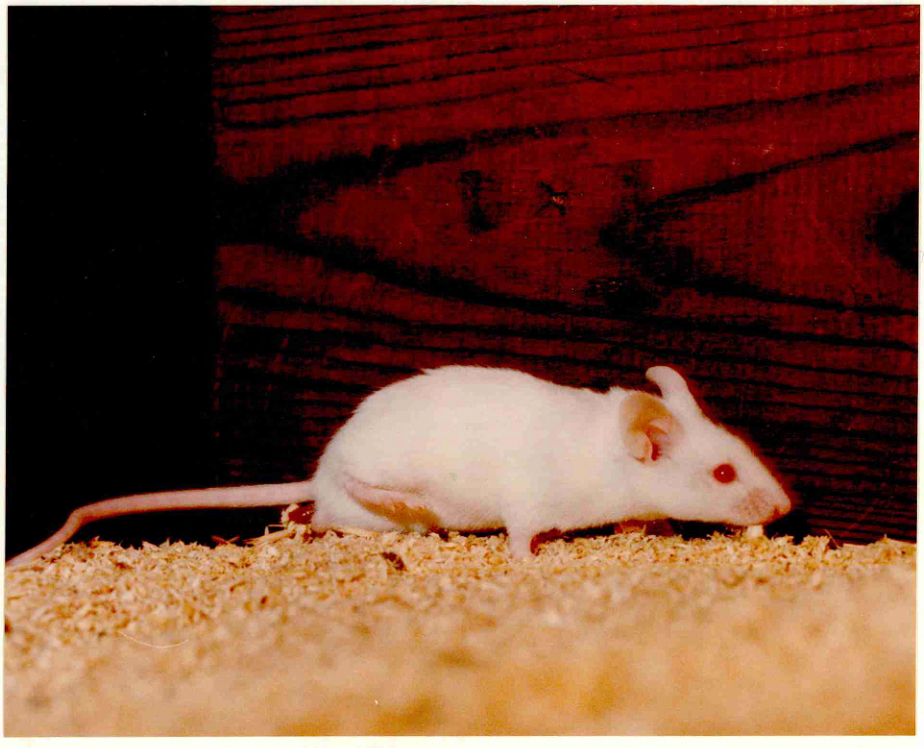


Fig. 2.5 Subject exhibiting locomotor behaviour

Fig. 2.6 Subjects exhibiting sniffing, head-lifting and rearing

Fig. 2.7 Subject exhibiting grooming

Self-grooming is included as another activity which is exhibited frequently by developing pups, and consequently has received some attention in rats (Bolles 1960) and mice (Fentress & Stilwell 1973). Grooming has even been used as an indication of emotional response (Robinson 1963). Included in this activity are licking and scratching (Fig. 2.7). In a developed form, this behaviour involves licking the forepaws and then using these to clean the head region. High frequency hind limb scratching of the back and head is also common and this begins in a rudimentary fashion after only a few days.

Suckling is an obvious and common form of behaviour which physically unites the mother and offspring in developing mammals (Fig 2.8).

In common with Lehrman (1961) the three terms, 'nursing', 'suckling' and 'suckling stimulus', will be used as follows in this study :

Nursing is the behaviour displayed by the mother which allows access to the nipples by the pups.

Suckling is the activity of the pups sucking the nipple. In scoring this activity, I have also included the active approach of the pup in nipple-seeking. This has been done because of the difficulty experienced in determining when nipple-seeking has been terminated and suckling has begun in young pups, partially hidden from view by the mother.

Suckling stimulus is the sum of the stimuli provided to the mother by the pup(s) suckling.

Eating solid food and drinking water, once again, represent activities which are only possible at a certain stage of development and for this reason are included as a measure of this stage. Both activities precede

Fig. 2.8 Subjects exhibiting suckling

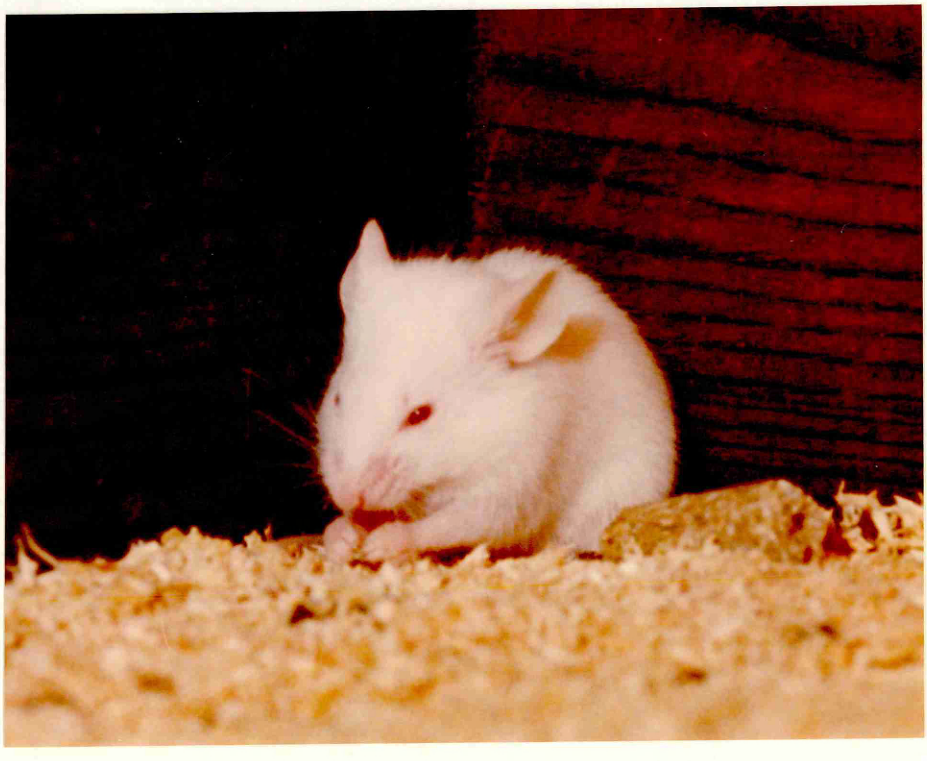


Fig. 2.9 Subject eating solid food

the stage of weaning. The eating of solid food begins with the pups finding particles of food on the floor of the cage which has crumbled from larger pellets during adult feeding (Fig. 2.9). Eventually they are able to feed from the pelleted food, deposited in the lid of the cage.

These activities were all important aspects of behaviour which were also easily noticed and easy to record. It was possible that the amount of time spent in them by the subjects could be affected by social factors of the sort under consideration in this project.

(iii) Experimental Techniques

1. The Research Approach. When recording rodent developmental behaviour there are different logical approaches to take. One is the non-manipulative and descriptive approach. This was used by Bolles & Wood (1964) whilst investigating the ontogeny of behaviour in rat pups. It provides estimates of the proportion of time the subjects are engaged in different kinds of activities. The researcher is limited to the observation of behavioural patterns which the animals actually do exhibit rather than the behaviour which the animals can exhibit. One is, therefore, measuring the development of displayed behaviour rather than the development of behavioural capabilities. This is a common approach to take with any study of animal behaviour because it is easily conducted and, as mentioned earlier, reduces the effect of the observer on the subjects. Laboratory mouse investigations are no exception (Williams & Scott 1953). Much of the earlier work described in this chapter has reflected this approach, but it is not the only approach taken.

Fox (1965) and Wahlsten (1972) subjected laboratory mice to a barrage of reflexological tests on a daily basis in order to assess behavioural capabilities. Responses made by the pups were measured on a scale to show the intensity of the response. My preliminary researches included a repeat of some of Fox's work, but at this stage is was more to study the effect of the method rather than to confirm or refute his results. The following tests were administered on a daily basis :

Righting reflex - Does the pup return to a stable position, with all four feet on the ground, when placed on its side ?

Forelimb placing response - Does the pup raise and place its foot on the surface of an object when the dorsum of its foot is placed against the object's edge ?

Hindlimb placing response - As above, but with the hindlimb.

Acceleration righting response - Does the pup turn in mid air and land on all fours when released from an inverted position ? (This response is tested over a sheet of foam rubber.)

Rooting reflex - Does the pup crawl forwards when the face region of the head is stimulated by bilateral pressure administered with the experimenter's finger and thumb ?

Vibrissa placing response - Does the pup raise its head and extend its forelimbs to contact an object when it is lowered by its tail until its vibrissae make contact with the object ?

Negative geotaxis - Does the pup turn and climb up a 45 degree, sandpaper-covered slope when placed on the slope in a downward direction ?

Cliff drop aversion - Does the pup turn and crawl away from a 'cliff' edge when placed with its forepaws and head over the edge ? (This response is also tested above a sheet of foam rubber.)

Auricular startle response - Does the pup show an immediate startle reaction when a retractable ballpoint pen is 'clicked' 5cm from its head ?

After running these tests on three litters of mice, the following observations were made :

(a) The above tests could all be carried out with ease.

(b) It would be possible to subject different groups of mice to different environmental variables (such as litter size, maternal experience and paternal presence) and see their effect on the responses made by the pups to the reflexological tests. In this way the effect of the variables on behavioural development could be measured.

(c) The recording of the intensity of the response, on either a 5 point or a 10 point scale, was a subjective measure and difficult to carry out in practice. Whereas intra-observer reliability could possibly be achieved, it is questionable whether inter-observer reliability could. This is obviously important when comparing the results of different studies.

(d) This series of tests represented, for single pups, a demanding and stressful situation which would surely have its consequent behavioural repercussions.

(e) It is possible that manipulation of the pup interfered with its social relationships with littermates and parents. Considerable disturbance to the rest of the litter and parents was caused by the removal and replacement of the test pup. It is also unlikely that the adults' behaviour would remain unaffected by this daily intrusion into their normal activities. This, in turn, could affect the pups' behaviour.

My own view, after the completion of these tests, was that they could provide interesting information about the way in which the pups' behavioural capabilities changed with age, but that the mode of testing was interfering with the very aspect of behaviour which I wanted to measure. The environmental variables that concerned me did not include that of human interference. It was to be hoped that the findings of this project would have some external validity (where inferences and generalizations from the experiment sample would apply to the wild situation) as well as internal validity. The reflexological tests constituted a far too dominant influence in the pups' daily set of experiences to be continued. Interference of this nature is known to cause changes in subsequent behaviour. Priestnall (1973a) showed an immediate effect of pup handling on maternal behaviour. There was increased licking by the mother of handled pups in the hour after treatment. Lee & Williams (1974), whilst conducting similar researches in rats, claimed that more lasting changes in maternal behaviour occurred. From this stage onwards only the non-manipulative, descriptive approach to recording behaviour was continued and it was felt that its disadvantages were small in comparison with the disadvantages of the reflexological test approach.

2. Methods of Recording Behaviour. There are widely differing approaches to recording behaviour and two factors predominate in determining which to use. One is suitability for later statistical analysis and the other is the availability of equipment. Ideally, the most effective recording system would monitor accurately the behaviour of the subjects over a 24 hour period, each day for the entire recording period. Since this is neither practicable nor possible, a method of sampling the behaviour of the subjects must be adopted.

Two rodent studies exemplify possible methods to adopt. Priestnall (1970) studied laboratory mice using a 10 minute observation session on every alternate day. During this time he noted all changes of behaviour made by the subjects. Whilst carrying out further investigations, he used extended observation sessions of four hours. These involved a technique whereby a number of different cages were observed serially. The observer moved from cage to cage and noted on a prepared record sheet the category of behaviour displayed by each subject at the time of observation. The cycle was completed in approximately four minutes. This was continued for four hours and therefore produced a maximum of 60 scores for each subject. Elwood & Broom (1978) studied gerbil pups on days 0, 1 and 2 and then on alternate days until day 24. Each family group at each litter age was observed for a total of 30 minutes in four separate seven and a half minute sessions. There was at least one 10 minute interval between successive sessions. The subjects were observed for 10 seconds and a note of their behaviour was made on a prepared record sheet in the following 5 seconds. This meant that a maximum score of 120 could be obtained each day.

A distinction will now be made between two different techniques of sampling behaviour; namely 'period occurrence' and 'instantaneous sampling'. Hinde et al. (1964) and Mitchell (1968) are workers who have used the period occurrence technique. The technique involves the observation of behaviour for short predetermined periods of time. These periods would each have a short duration; for example 15 seconds; and they would be grouped to provide a total of about 20 sample periods in succession. The period occurrence technique involves the recording of the occurrence or non-occurrence of certain predetermined and objectively defined forms of behaviour during each one of these periods of time. Each form of behaviour will only be recorded once and so a measure of frequency is not made. This is often referred to as 'one-zero' sampling. Altmann (1974) pointed out that most users of the technique were attempting to sample states of behaviour (that is a behaviour with a duration, such as being in a nest) rather than events (an instantaneous recording of the onset of behaviour, such as entering a nest). When the duration of forms of behavior are being recorded, a measure of duration is needed and so the recording session must be long enough to obtain an estimate of the length of the longest duration. This technique has been criticized on the grounds that it is used with a too short length of time being allocated to the sampling period. Arrington (1943) has provided a critical review of this sampling method. Altmann (1974) continued by decribing the technique as one which ignored the important differences between frequency (dealing with events) and duration (dealing with states). Mitchell (1968) recognized that the technique imposed an upper limit on the number of times a form of behaviour could occur, which might be far less than the actual frequency. Altmann (1974) agreed with this criticism and for this reason, did not recommend the use of this technique. The point was made that the technique of period occurrence was a measure of the frequency of intervals

that included any amount of time engaged in a behavioural category, and not the percentage of time spent in that activity. It did not, the claim continued, accurately measure either duration or frequency of behaviour.

An alternative to this is the instantaneous sampling technique. Here the observer scans and then records an individual's activity at preselected moments in time, such as every minute on the minute throughout the hour. This was adopted for use by Richards (1966b) whilst studying golden hamsters. Simpson & Simpson (1977) have pointed out disadvantages of this approach. It is possible for the scan to miss behaviour which is of very short duration. Simpson & Simpson (1977) also provided evidence, indicating that this technique does not provide information on the frequencies of the onsets or offsets of behavioural activities. Tyler (1979) added that with a complex set of behaviours it would be conceivable that the observer would bias the recording towards the more obvious behaviours since, with several activities occuring simultaneously, the less obvious might be ignored. It was approved by Altmann (1974), however, for recording easily and quickly, distinguishable behaviour, on the grounds that it genuinely records states of behaviour and provides an unbiased estimate of the amount or percentage of time a subject devotes to particular activities. The percentage time is estimated from the percentage of samples in which the particular activity was recorded. Dunbar (1976) compared period occurrence to instantaneous sampling for estimating the proportion of time subjects were engaged in grooming. The former gave poor estimates whereas the latter always gave reliable estimates.

On the above grounds it was decided that the instantaneous sampling technique would be the most desirable to use. All 12 behavioural

categories would be recorded simultaneously and for this reason, only one subject animal was observed during any one recording session. This obviously neglects the recording of behavioural synchrony and interactions between animals. The decision to adopt this 'single subject animal' approach was only made after investigating whether a single pup's behaviour was typical of the entire litter. It was apparent that during the first 12 days of life the pup's behaviour was synchronized with the rest of the litter. When one pup was suckling, it was highly likely that the rest of the litter was suckling. Even periods of active movement and rest were experienced by the litter at approximately the same times. Later, when synchronized behaviour was no longer apparent, one pup's behaviour appeared to typify the other pups' behaviour in terms of the frequency and duration of activities. Such focal animal sampling, rather than group sampling, has been used by many authors including, for example, Rosenblum & Kaufman (1967) and by Doyle et al. (1969). Intra-litter variations will not affect the data with this technique. The same focal pup was observed day after day and for this reason was marked to aid identification. Since recording did not commence until day 10, when hair had grown, this was an easy operation and was initially carried out with black candle-maker's dye. Porter & Doane (1978) and Porter & Wyrick (1979) claim that a rapidly drying paint on the back of rodents has no noticable effect on social interactions involving adults and/or infants of either sex.

The next aspect of recording to be determined was the sample session length. Observer fatigue; which Altmann (1974) claimed was influenced by familiarity and experience, the number of behavioural categories being recorded and the rapidity of behavioural change; was a major factor which determined this length. The behaviour of the pups

did change rapidly and for this reason it was measured every 5 seconds. After experimenting with different length sessions, a 10 minute session was adopted, but this was eventually reduced to 8 minutes and 20 seconds in order to provide a maximum of 100 scores per day for each of the 12 behavioural categories.

The actual recording of behaviour is important and obviously must not interfere with the observation of the subjects. Priestnall (1970) used a microphone and tape recorder with a two-channel event recorder. During each recording session, each behavioural change was noted verbally and a key depressed on the event recorder. By later running back the tape, a measure of duration and frequency of behaviour was obtained. After experimenting with a tape recorder and, later, an assistant, I eventually settled on a different method whereby the behaviour was observed on the turn of each 5 seconds. No behaviour was recorded which occurred in the interval between two successive signals. The time was indicated by a low-volume 230Hz signal produced by a piece of electrical apparatus. When the signal sounded, there was no noticeable effect on the subject's behaviour. The signal was started 10 minutes before the recording session commenced in order to prevent any possible startle reaction. During the recording session, a tick was entered on a check sheet for each of the 12 behavioural activities exhibited at each scan. This was done during the interval between two successive signals. In this way there was no conflict between the two distinct activities of observing and recording. With practice, there was sufficient time to make all necessary records in the time available, even when the behavioural changes were particularly rapid.

With this system, 12 behavioural scores (representing the 12 categories of behaviour) were obtained each day for each focal subject pup. Although there are activities which are mutually exclusive, such as suckling and eating solid food, some activities do not preclude others from also being exhibited. In most cases, several activities would be exhibited at each scan. The maximum score for each of the 12 activities was 100. Such a score of 100 would only be obtained if the subject pup exhibited that category of behaviour on every one of the 100 sound signals. Once the entire recording period, up to day 25, was complete, it was possible to analyse all of the daily scores in each category. Consequently the data were objective, numerical and ready for statistical treatment.

Other measures were taken at the end of the pre-weaning period and these are explained in Chapter 3, which deals with the main experiment.

3. Time of Day For Recording. Only if one wished to assume that diurnal and nocturnal variation in behaviour was zero or negligible, would one be justified in recording behaviour at different times of the day. Ashby (1972), Beck (1963), Lockard (1963) and Wimer & Fuller (1968) have indicated that a definite rhythm of activities takes place during each period of 24 hours, which cannot be ignored in laboratory rodents. Tuffery (1967) claimed that the laboratory mouse breeds well under a controlled artificial light cycle of 12 hours on and 12 hours off. This cycle was adopted in common with many other rodent researchers.

Elwood & Broom (1978) carried out their gerbil recordings during the latter half of the dark phase of the cycle. Although Priestnall (1970) recorded laboratory mouse behaviour at different times of the day, he always recorded during the dark phase. This is understandable since the animal is active at night and when recording is carried out under red light (a wavelength which does not appear to stimulate these animals' retinal cells), natural dark phase behaviour can be observed without the subjects seeing the observer.

In my own work, observation lighting consisted of a red 60 Watt bulb which was placed over the subjects' cage, and which was operated by a time switch to come on one hour before the recording commenced.

The most convenient time for me to regularly record behaviour on a daily basis was early in the morning and so the light phase began at 0900 hours. The light phase ran from 0900 hours until 2100 hours and the dark phase from 2100 hours to 0900 hours. By recording behaviour between 0700 hours and 0900 hours each day, I was able to take full advantage of the latter part of the dark phase.

As a result of completing the early experimental work, outlined in this chapter, sufficient basic and foundational knowledge and skills were established to proceed to the main experiment. This is outlined in Chapter 3.

Chapter Three

THE MAIN EXPERIMENT : MATERIALS AND METHODS

CHAPTER 3

THE MAIN EXPERIMENT : MATERIALS AND METHODS

The purpose of this experimental work was to establish the effect of three variables on mouse pup behavioural development.
The three variables were :

1. Litter size
2. Maternal experience
3. Paternal presence

Reasons are given for the selection of these variables in Chapter 1. In this chapter a full description is given of the materials and methods used, both for experimental work and for statistical treatments. I was not only interested in the specific effect of the three variables, but also in the effect of any interactions between the variables. To satisfy these requirements, a three factor factorial design was adopted.

1. Experimental Design

A design was required which would reveal whether or not each of the three independent variables had any systematic effect on the behaviour of the developing mouse pups. A factorial design with equal numbers of observations per cell was employed, with a fixed effect analysis of variance used to test the effects of the variables. The choice of this design presented several advantages. The multi-factorial design has been advocated on the grounds that if factors are studied concurrently, the total number of subjects needed for experimentation can be reduced (Guidelines For The Use of Animals in Research 1981;

Still 1982). The more traditional approach, of studying the effects of different treatments in different experiments, does not allow the effects of interactions between treatments to be investigated.

In this study it was considered desirable to evaluate the effect of interactions. As explained in Chapter 1, the variables in question would be pertinent in a wild situation where there would be the opportunity for combinations of these factors to exert their effect. A realistic design, therefore, would necessitate the inclusion of such combinations of factors. Petrinovich (1981) has commented on the suitability of the multi-factorial design and the use of the analysis of variance for research of this nature.

Table 3.1 shows the experimental design incorporating the three variables. A 2 x 2 x 2 design was used with four sets of replicates. This represented 32 litters with a total of 144 pups. One set of results was obtained for each litter.

Two widely different litter sizes were chosen. Small litters contained two pups and large litters contained seven pups. Two pups in a litter provided a sufficient stimulus for continued lactation and enabled observations to be made on any interaction which might take place between the subject pup and a littermate. A litter size of at least seven pups could be expected from most parturitions and seven was therefore chosen as the size of the large litter. On day 2 (the birth day representing day 0), the litter sizes were obtained by culling. Delaying until day 2, avoided litter disturbance during the very sensitive early postpartum period when parents are more likely to display infanticidal behaviour.

Table 3.1

Factor Arrangement Table

Eight different arrangements exist within one replicate set. Results from four sets of replicates were obtained.

Litter Size	Parity of Mother	Presence of Father
Two Pups	Primiparous	Absent
		Present
	Multiparous	Absent
		Present
Seven Pups	Primiparous	Absent
		Present
	Multiparous	Absent
		Present

The second variable was maternal experience. Again two forms of this variable were chosen. The first was the primiparous state, and the second was the multiparous one. The mother never experienced a change in breeding partner which meant that the parental experience of the father was always the same as that of the mother he accompanied. This, consequently, must be borne in mind when evaluating the effect of the variable.

The third variable was that of paternal presence. In half of the experimental cages, the father was present and in the other half he was removed. The father was removed, as is explained in Chapter 2, on day 0, just after the litter was born. Removing the father on day 0 prevented copulation and the onset of gestation during the postpartum oestrus. In the event of any experimental subject mortality, the litter was not included in the analysis, and any results already obtained were discarded. Another litter was then introduced to make good the loss. If a mother became noticeably pregnant during the recording period, the results obtained from that litter were not used in the analysis since the changed endocrinal state during pregnancy could affect her maternal performance. Again, under these circumstances, another litter was substituted to ensure that complete sets of data were obtained.

2. Preparation For Experimentation

The subjects and their housing conditions are described in Chapter 2. Food and water were provided ad libitum. As soon as the female became noticeably pregnant, the prospective parents were placed into clean cages with fresh sawdust. As explained above, once the litter was born, adjustments were made to the litter size and, where necessary,

the father was removed. From this point through to day 9, the family group was not interfered with.

On day 9 the father was marked to distinguish him from the mother during observation sessions. On the same day a focal subject pup was chosen and marked to distinguish it from the rest of the litter. Again the subject pup had to be easily recognizable from the rest of the litter during the observation and recording sessions. The same pup was used as an observation subject each day that recordings were made. Care was taken to help ensure that equal numbers of either sex were represented as subjects; but apart from this consideration, pups were chosen randomly. Since the sex of the subject would constitute an additional independent variable, this was dealt with by using equal numbers of males and females. Subjects were marked on the back of the neck with a black, spirit-based, candle-makers' dye and was reinforced every two days. These procedures were preparatory to the observation sessions which began on day 10 and which are described below. Identification marking and the use of a focal subject pup are discussed in greater detail in Chapter 2. The cage and its occupants were interfered with during the experimental period only when absolutely necessary. Such interference was limited to that which has already been stated, plus the daily feeding and water bottle refilling.

3. Observations And Collection Of Results

Recordings were made of the subjects' behaviour during their pre-weaning development, starting on day 10 and continuing to day 25. Twelve different activities were monitored on a daily basis. These are shown in Table 3.2 and are described in Chapter 2.

Table 3.2

Categories of Behaviour

(1) Presence of the pup in the nest

(2) Pup's head within 1cm. of any other member of the litter (proximity to littermates)

(3) Pup's head within 1cm. of the mother (proximity to mother)

(4) Pup's head within 1cm. of the father (proximity to father)

(5) Locomotor behaviour (crawling, walking, running, digging)

(6) Exploratory behaviour : Sniffing

(7) Exploratory behaviour : Head-lifting

(8) Exploratory behaviour : Rearing

(9) Grooming behaviour: (scratching, licking)

(10) Suckling (including active nipple-seeking)

(11) Eating solid food

(12) Drinking water

[Definitions of behaviour categories are provided in Chapter 2.]

At the end of each day's recording, the marks in each of the 12 categories were summed to establish the proportion of the maximum 100 marks which had been obtained. With recording beginning on day 10 and ending on day 25, 16 days' results were obtained for each litter. The 16 scores were reduced to eight by calculating the means of each two successive days' results. This tended to regulate the trends which occurred with time. Some irregularities were caused by a 'flip-flop' effect occasionally noticed in some of the data; where, for a particular activity, a maximum score was obtained for one recording session and a zero score obtained for the next. Pooling of data over two days helped to overcome this.

Day 30 Measurements

Litters were subjected to their particular treatments until day 30, although daily recordings of the 12 activities were completed on day 25. This extension of five days was intended to allow the enhancement of any differences which were due to the different treatments before final measurements were taken on day 30. Two types of measurements were made on day 30. An open field test was conducted on the entire litter, not just on one subject pup; after which body weights of the entire litter were measured.

Open Field Tests. The open field test was conducted to investigate the effect of the treatments on ambulation and defaecation scores. No tests were conducted during the earlier pre-weaning period in order to minimize pup disturbance. Such a test would subject the pups to a treatment which would far exceed that considered desirable in the non-manipulative and observational stage of the study.

The apparatus used is shown in Fig 3.1. The apparatus was constructed from a hardwood base and a hardboard wall. The base was composed of a circular arena, marked into 19 compartments of equal area. Twelve of these were peripheral compartments adjoining the outer wall, and the other seven were inner compartments. Each subject pup was placed into the arena in turn and three types of measurements were taken over a five minute period. The following were recorded :

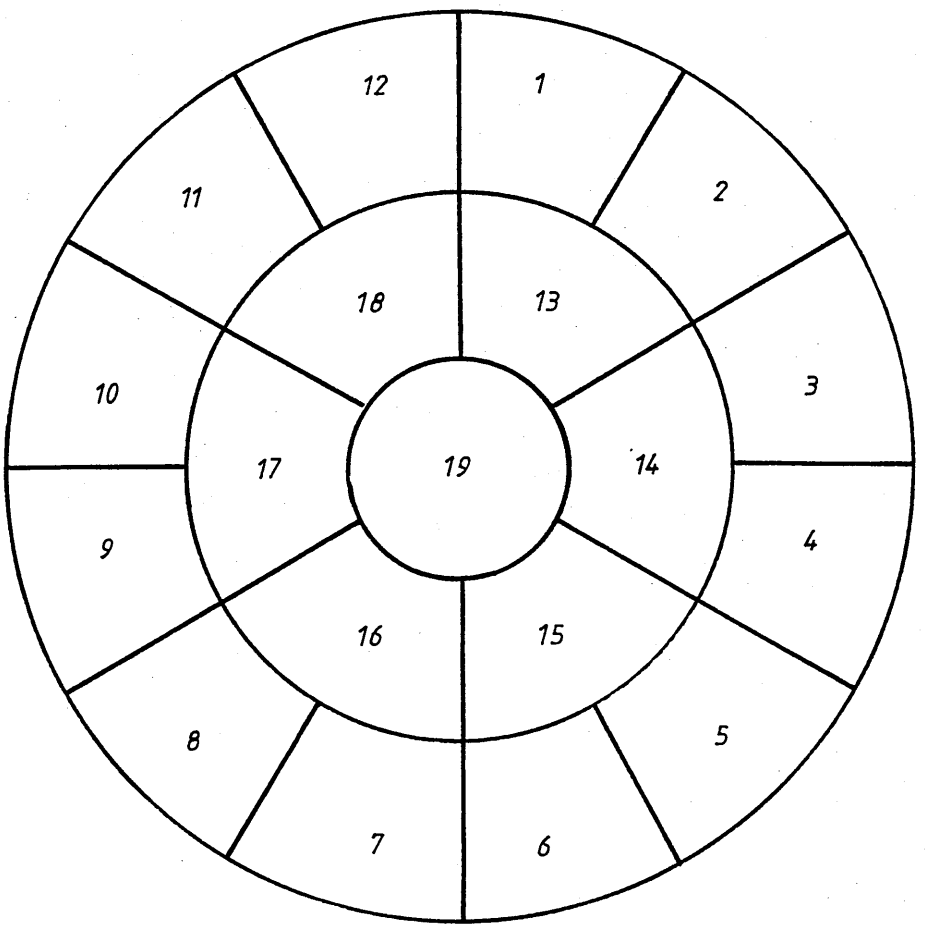
(a) Number of peripheral compartments entered by the subject (a compartment entry was scored when all four legs of the subject had crossed the compartment boundary).

(b) Number of inner compartments entered by the subject.

(c) Number of faecal boli produced by the subject.

Each subject in turn was placed in one of the peripheral compartments and the five minute period timed from the moment of release. After the period of testing, the floor was rinsed well with tap water and wiped dry with a clean paper towel in order to eliminate or reduce scents which could interfere with the next subject's behaviour. Whittier & McReynolds (1965) showed that persistent odours were left in open field apparatus by laboratory mice and that these odours constituted an attractant to other mice subsequently placed in the same apparatus. LaBarba & Hodge (1970) resorted to using vinegar solution in an attempt to remove the odours. All of the open field tests were conducted half way through the light phase of the day/night cycle, when ceiling fluorescent strip lighting was on.

The open field test was considered an easy to administer measure of the effects of the experimental treatments on the subjects' behaviour at the close of their pre-weaning period. The purpose of the test was to make intra-experiment comparisons, rather than to determine



Diameter of base: 48cm.
Height of internal wall: 32.5cm.

Peripheral compartments: 1-12
Inner compartments: 13-14

Fig.3.1 Open field apparatus.

particular emotionality levels. It was hoped that the three open field measurements would be sensitive to the different experimental treatments received by the subjects. No attempt was made to measure the 'emotionality' of the subjects, for a number of reasons. LaBarba, Lutz & White (1968) recommended caution when applying the open field criteria to establish emotionality across rodent species and across strains. Steng (1971) found wide inter-strain differences in inbred laboratory mouse open field behaviour. Walsh & Cummins (1976) produced a critical review of the open field test and expressed concern at the lack of conformity in both procedure and results. Whimbey & Denenberg (1967) expressed the view that the open field ambulation measure obtained from the first of a series of tests was not particularly meaningful in establishing the emotionality of the subjects, and that it was necessary to test over several days and use summed scores. Levine et al. (1967) and Williams & Russell (1972) found that the first day ambulation scores were higher than those obtained on subsequent days. Whimbey & Denenberg (1967) found that some first day measures provided the opposite results from those expected, or failed to provide differences in the results when large differences were expected. They further maintained that high ambulation scores on the first day were indicative of high emotionality reactivity, whereas high ambulation scores during subsequent days' testing reflected low emotional reactivity.

The question of validity of the open field test to determine the 'emotionality' of the subjects has been raised. The connotations of the term caused concern for Tachibana (1980) who preferred to use the term, 'emotional reactivity to a novel situation'. Russell (1971) questioned whether the concept of 'emotionality' was an adequate one for the use it was being put. Problems are associated with attempts to measure the internal behavioural state of an animal. It is one

thing to measure ambulation and another to suggest that ambulation scores are a measure of the stability or emotionality of the subjects. Some ambulation in my own open field tests were caused by highly erratic and excited behaviour; not the sort of behaviour which brings to mind the term, 'stable'. Archer (1973) suggested that ambulation scores have little descriptive validity as a measure of confidence and exploration. Alternative forms of behaviour which characterize high emotionality would be immobility and escape behaviour and this casts doubt on the view that ambulation scores are adequately indicating a particular emotionality response. Daly (1973) criticized the conventional association between a high ambulation score and a low emotionality state by claiming that any small rodent that unhesitatingly entered a brightly lit, novel environment must be "pathologically fearless". Such an animal could not be considered to be 'better adjusted', nor would the experience which brought about such a state be considered 'beneficial'. Archer (1973) criticised the emotionality concept on the grounds that there was a lack of relationship between the different measures of it, but suggested that the open field test could be used without reference to emotionality. I decided to use the test in this way in my own experimentations. I was also interested, however, in the extent to which subjects were prepared to depart from their wall-seeking tendency (Fredericson 1953). Since this could be established through the 'inner compartment' ambulation score, two ambulation scores; peripheral and inner compartment scores; were obtained instead of just one.

Body Weights. Immediately after each open field test was conducted, the subject pup was weighed. Body weight, which is one measure of physical development, was recorded to see whether any particular treatments or combinations of treatment were associated with elevated or depressed body weight scores at this age. Although the principal

purpose of this study is to investigate behavioural development, the measurement of body weights was conducted for two reasons. First, body weight provides a simple, if crude, measure of physical development, and has been used as such by many other researchers. Second it is quickly and easily ascertained.

4. Treatment of Data

Data, obtained from the continuous daily observations and from the day 30 measurements, were subjected to a three way analysis of variance (ANOVA) with litter size, maternal experience and paternal presence as the three main factors.

The advantages of ANOVA are well known and particularly relevant to this design where it has been necessary to test the effect of three independent variables at the same time and to look for significant interactions between the variables. In behavioural research, as in other scientific areas, it is considered theoretically important to study both main effects and interactions (Petrinovich 1981). The condition of distribution normality must be satisfied before using a parametric test (Siegel 1956). Upon inspection, the distribution of the daily observation data appeared skewed. In order to help satisfy the normally distributed population condition, all scores obtained from the daily observations, which happened to be out of 100 (and had not been converted to percentages for the purpose), were transformed using an angular transformation (ARCSIN) before being subjected to the ANOVA (Campbell 1974; Kirk 1968). These same activity scores involved measures taken over a period of time with eight age levels. Initially age was included in the ANOVA as a fourth independent variable, but this resulted in some complex interaction effects with age that were

difficult to interpret. For the following reasons, I considered it preferable to treat age as a covariate (Kirk 1968; Scheffé 1959).

1. Age is a different type of variable from the other three independent variables. The former is a quantitative factor, whereas the latter are qualitative factors, and it is valuable to distinguish between the two. Treating age as a covariate achieves this.

2. It is possible to clarify the effects of the three main independent variables, including their interactions with each other, by treating age as a covariate.

3. Treating age as a covariate allows general age trends to be recognized easily for the behavioural activities, and it is possible, by additional analysis (see Chapter 4) to detect any interactions between the independent variables and age.

An alternative to this would have been to adopt the more classical approach of examining separately the effects of the three social factors on behaviours at the various ages. This would have allowed interactions with age to be investigated without creating a too complex analysis. Such an approach, however, would have prevented the study of interactions between the three social factors.

For the day 30 scores, the analyses were carried out on litter means rather than on the separate offspring scores. This practice, which causes the statistical test to become more stringent, was adopted in order to eliminate the problem of correlation of observations within litters. Abbey & Howard (1973) have critically commented on the practice of calculating from the measurements of individual pups rather than from the means of the different litters.

Summary tables for all of the analyses of variance are presented

in Chapter 4. These show the sources of variance, the mean squares (MS), the degrees of freedom (df) and the F ratios. The probability levels (P) are given when there are significant effects. In interpreting the summary table, higher order interactions were tested against the residual. Where these were not significant, the next level interactions were tested against the residual. This was continued until a significant effect was found or until the main effects were tested against the residual. Where there was a significant effect for an interaction, any related lower order interaction or related main effect was not tested against the residual, but against the significant interaction (Lindner 1979; Scheffé 1959). In the event of having two significant two-way interactions, the main effect was tested against the interaction which provided the more stringent statistical test. Type 1 errors are less likely to occur with this practice than when all main effects and interactions are tested against the residual, regardless of the existence of significant interactions.

Since the ANOVA is designed to establish whether or not the independent variables have significant effects, and not what the effects are; a post ANOVA multiple range test was administered to compare means in order to see where significant differences occur. In common with Priestnall & Young (1978), the test used was the Scheffé test, applied at the 5 % level of significance. This test utilizes F tables in order to compute a critical difference (Bruning & Kintz 1977).

Graphical presentation of results, discussions and conclusions are provided for each of the three main variables and their interactions, in Chapters 4 and 5.

Chapter Four

RESULTS FROM THE MAIN EXPERIMENT

Chapter 4

RESULTS FROM THE MAIN EXPERIMENT

This chapter contains an age analysis table (Table 4.A) and 15 ANOVA summary tables with Scheffé test results (Tables 4.1 to 4.15). There were 12 behavioural categories measured repeatedly during the pre-weaning period and four, day 30 measures. 'Drinking water' was an activity not included in the analysis because it was observed so infrequently. For the other 11 of the 12 behavioural categories, graphs are provided with means for each main factor averaged over the other main factors, and plotted against a pup age scale (Figs. 4.1 to 4.11). Each number on the 'pup age' axis refers to the first day of the pair of days from which each result had been obtained. Two successive days' results were averaged to obtain a single score. For example, '18' refers to the overall score obtained by averaging the two scores from days 18 to 19. Histograms are provided for the four measures taken on day 30 (Figs. 4.12 to 4.15). All graphs are based on untransformed data. Following each ANOVA summary table and accompanying graphs, a brief description of the statistical test results is given. For the behavioural activities measured each day, comments are included on trends associated with the increasing age of the pups. These comments are based on the age analyses and Figs. 4.1 to 4.11. Significant correlations, between age and the incidence of display of a behavioural activity, are indicated. Table 4.A provides a summary of the age analyses and shows significant age effects for the measures, as revealed by the analysis of covariance. These are general age effects for each behavioural activity. In addition to this, Table 4.A shows how the age effects are distributed between the different treatments. Pearson product moment correlation coefficient analyses were carried out between individual pup scores for behaviours under each separate experimental condition and pup age. The effects of

Table 4.A Age Analyses for Main Treatment Effects

Values for F, df, and P are shown for the Covariate analyses

Values for r (correlation coefficient) and P (two-tailed) are shown for the Pearson Correlation analyses

MEASURE & Covariate values		TREATMENTS & Correlation values					
		2	7	Pr	Mu	M	F
IN NEST	r	-0.232	-0.476	-0.502	-0.228	-0.503	-0.310
F=55.527 df=1,247 P<0.001	P	<0.05	<0.001	<0.001	=0.01	<0.001	<0.001
PROXIMITY TO LITTERMATE	r	-0.331	-0.447	-0.402	-0.304	-0.453	-0.187
F=55.145 df=1,247 P<0.001	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
PROXIMITY TO MOTHER	r	0.180	0.061	0.029	0.221	0.115	0.132
F=3.508 df=1,247 NS	P	<0.05	NS	NS	<0.05	NS	NS
PROXIMITY TO FATHER	r	0.390	-0.118	-0.192	0.510		
F=3.502 df=1,123 NS	P	<0.001	NS	NS	<0.001		
LOCOMOTION	r	-0.150	-0.208	-0.155	-0.202	-0.218	-0.156
F=7.973 df=1,247 P<0.01	P	NS	<0.05	NS	<0.05	<0.05	NS
SNIFFING	r	0.185	0.372	0.248	0.289	0.435	0.114
F=35.822 df=1,247 P<0.001	P	<0.05	<0.001	<0.01	<0.001	<0.001	NS
HEAD-LIFTING	r	0.007	0.236	0.101	0.076	0.274	0.020
F=6.412 df.1,247 P<0.05	P	NS	<0.01	NS	NS	<0.01	NS
REARING	r	0.074	0.277	0.202	0.113	0.204	0.146
F=13.733 df=1,247 P<0.001	P	NS	<0.01	<0.05	NS	<0.05	NS
GROOMING	r	0.476	0.517	0.491	0.498	0.496	0.521
F=85.943 df=1,247 P<0.001	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SUCKLING	r	-0.087	-0.093	-0.190	0.012	0.040	-0.204
F=0.947 df=1,247 NS	P	NS	NS	<0.05	NS	NS	<0.05
EATING SOLID FOOD	r	0.346	0.501	0.492	0.343	0.539	0.399
F=89.451 df=1,247 P<0.001	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

2: litter size of 2 pups

7: litter size of 7 pups

Pr: primiparous mother

Mu: multiparous mother

M: mother only present

F: father also present

the main factors and their interactions are not discussed until Chapter 5, where other people's work is reviewed whenever it relates to my own results.

Analysis of Variance Tables

Tables 4.1 to 4.5 show the results of the analyses of variance. Where there is a significant difference for a main effect or for an interaction, the Scheffé test results are shown. This is to indicate which of the differences among the treatment means are significant. Any two means not underlined by the same line are significantly different. Any two means underlined by the same line are not significantly different. This convention was used by Duncan (1955). (Tables 4.1 to 4.11 are based on transformed data. In these tables, backtransformed means, expressed as percentages, are provided in parentheses.)

Abbreviations. The following abbreviations have been used in the tables:

L	: Litter Size	MS	: mean square
M	: Maternal Experience	F	: the F ratio
P	: Paternal Presence	P	: the probability level
df	: degrees of freedom (applied to the F ratio)	NS	: not significant

The two litter sizes are indicated by the numbers '2' and '7'.

2 : litter containing two pups

7 : litter containing seven pups

The two states of maternal experience are indicated by the symbols 'Pr' and 'Mu'.

Pr : primiparous

Mu : multiparous

Whether the father is present or not is indicated by the symbols 'M' and 'F'.

M : only the mother is present (father absent)

F : the father is also present (father present)

Combinations of these factors are also indicated by using the same symbols. For example, '7-F' refers to all of the subjects which have been raised in a litter of seven pups and with the father present; and '2-Pr-M' refers to all of the subjects raised in a litter of two pups, with a primiparous mother and with no father present. Since different combinations of the levels of the three independent variables are mentioned repeatedly throughout the text in Chapter 5, the same abbreviations are used there.

1. Time in Nest

The ANOVA and Scheffé test results are shown in Table 4.1, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.1a, 4.1b and 4.1c. Two significant interactions need to be noted. One is the interaction between litter size and maternal experience, and the other is the interaction between maternal experience and paternal presence.

In the former, the Scheffé test did not reveal any significant difference between means of combinations of the two factors, litter size and maternal experience. On a number of occasions during the analyses of data from this experiment, a significant difference was found with the ANOVA, but the Scheffé test indicated that no significant difference existed between any of the means. It is recognized that the Scheffé test is more stringent than some other multiple range

Table 4.1

Analysis of Variance of 'In Nest' Scores
with Scheffé Test Results
(Based on transformed data, with age as a covariate)

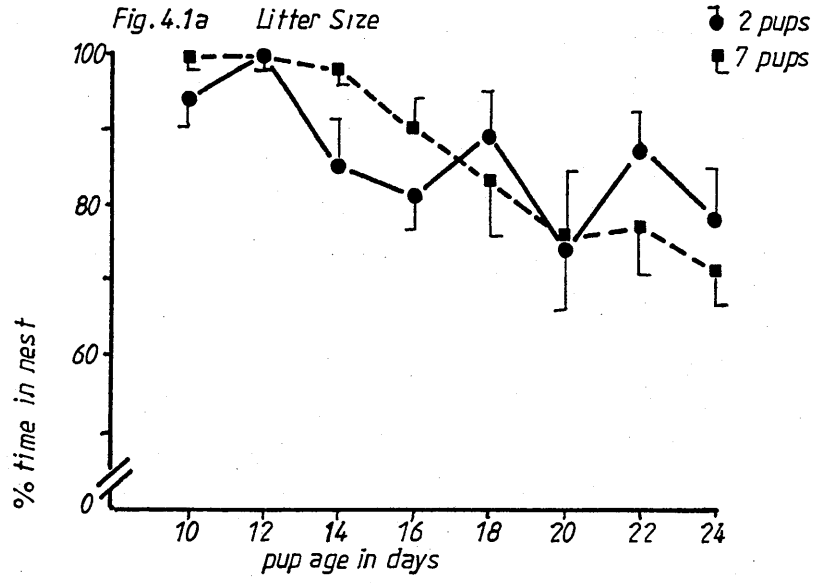
SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.017	0.038	NS
Maternal Experience (M)	1	0.200	0.451	NS
Paternal Presence (P)	1	1.157	2.885	NS
2-WAY INTERACTIONS				
L x M	1	0.443	4.461	< 0.05
L x P	1	0.027	0.271	NS
M x P	1	0.401	4.039	< 0.05
3-WAY INTERACTION				
L x M x P	1	0.002	0.021	NS
RESIDUAL	247	0.099		

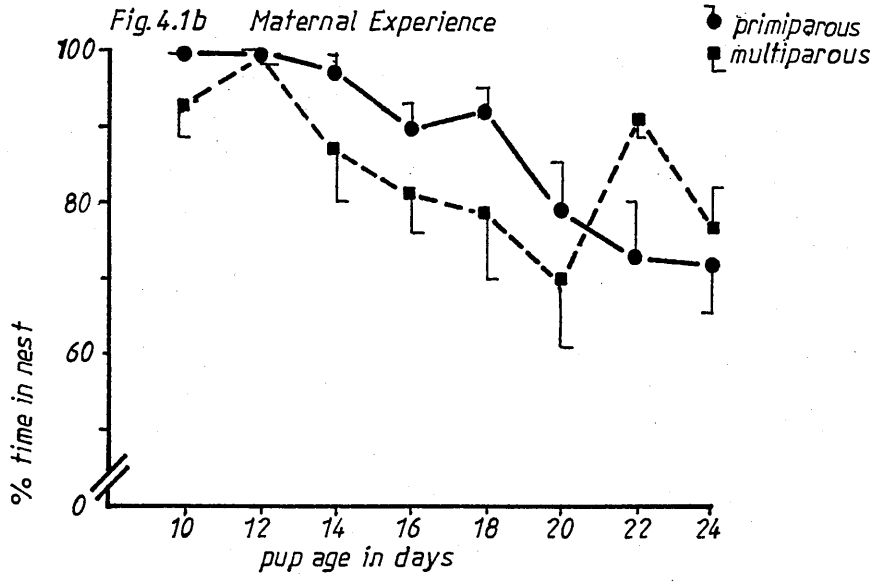
L x M

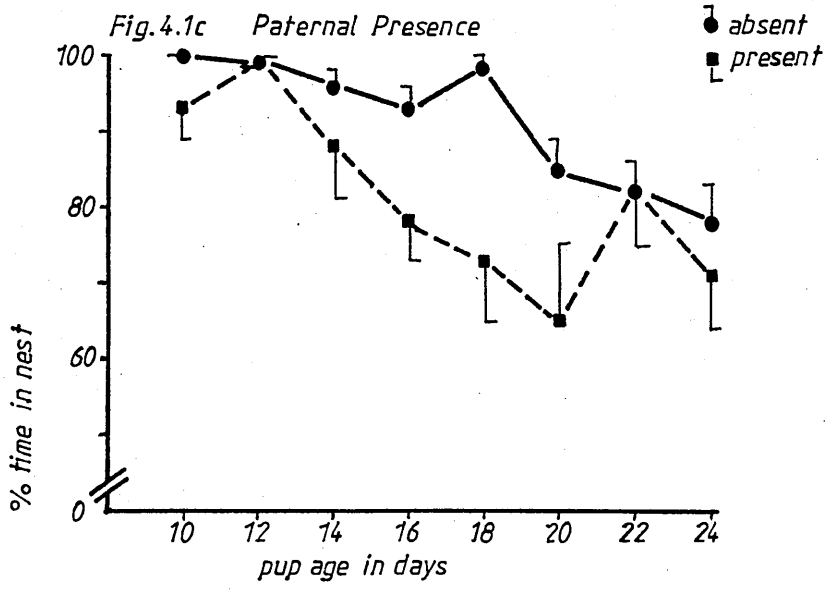
Combinations:	2-Mu	7-Pr	7-Mu	2-Pr
Means:	1.229	1.302	1.329	1.368
	(88.76)	(92.95)	(94.27)	(95.94)

M x P

Combinations:	Mu-F	Pr-F	Pr-M	Mu-M
Means:	1.172	1.307	1.363	1.386
	(84.92)	(93.20)	(95.74)	(96.62)







Mean percentage of instantaneous scans of pup in nest.
Standard errors are indicated.

tests (Bruning & Kintz 1977), which accounts for this occasional discrepancy. When it is not possible to identify which of the differences among the means may be considered significant using the Scheffé test, a comparison is made between the lowest mean and the highest mean. The greatest difference in this case was between the 2-Mu group which had the lowest mean and the 2-Pr group which had the highest mean.

In the maternal experience and paternal presence interaction, the Mu-F mean was significantly different from all of the other three means. This combination of a multiparous mother and the presence of the father resulted in the lowest mean score.

Trends Associated With Increasing Age of Pups

The analysis of covariance reveals a significant age effect ($P < 0.001$) and the correlation analyses show a significant negative correlation between this activity and age for all treatment conditions (Table 4.A). Figs. 4.1a, 4.1b and 4.1c show a very clear and expected downward trend. At the beginning of the recording period, pups were spending close to 100 per cent of the observation session in the nest. It is likely that by day 8 sufficient motor skill development had taken place to enable pups to leave the nest (Williams & Scott 1953), but until day 14, very little time was spent out of the nest. Remaining in the nest would provide a focal site for parental attention and also ensure constant contact with littermates. Retrieval back to the nest by parents was only very rarely observed, indicating that when pups remained in the nest, they did so on their own volition. The temperature maintenance of the pups during their early period of development would be helped by litter aggregation in the nest; where the smaller surface area to volume ratio of the group, compared to a single pup, would reduce the rate of heat loss from each pup. This

is clearly of some advantage to young mammals developing and maintaining homoiothermy. From day 14 onwards a general trend was observed of pups spending a smaller proportion of time in the nest. This downward trend was accompanied by an increase in exploratory behaviour and a shift from suckling to eating solid food (see comments below). Whereas most suckling took place in the nest, most ingestion of solid food took place out of the nest.

2. Proximity to Littermate

The ANOVA and Scheffé test results are shown in Table 4.2 and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.2a, 4.2b and 4.2c. Just one significant interaction was found and it was between litter size and maternal experience. The Scheffé test indicated that the 2-Mu group which had the lowest mean was significantly different from all of the other three combination means.

Trends Associated With Increasing Age of Pups

As the mouse pups began to leave the nest and move around the cage, a corresponding decrease in littermate proximity occurred (Figs. 4.2a, 4.2b and 4.2c). The analysis of covariance revealed a significant age effect ($P < 0.001$), and the correlation analyses showed significant negative correlations between this activity and age for all treatment conditions (Table 4.A). The difference between the two 'paternal presence' r values (Table 4.A) is likely to be primarily due to the 'father-present' mean scores showing an increase from day 22 onwards (Fig. 4.2c). It is possible that this upward trend resulted from some common focus of interest for the members of the litter, thus causing them to remain close to each other. Williams & Scott (1953) found that

Table 4.2

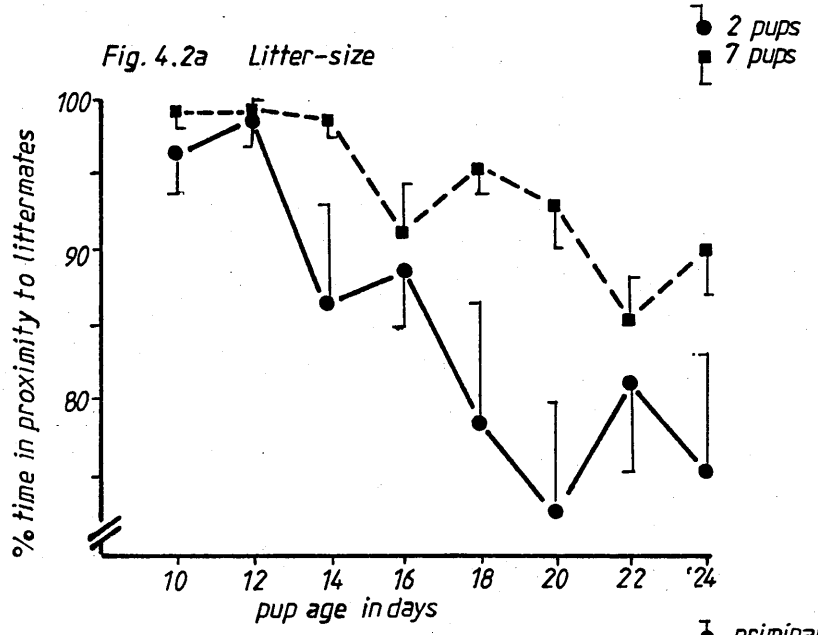
Analysis of Variance of 'Proximity to Littermate' Scores with Scheffé Test Results

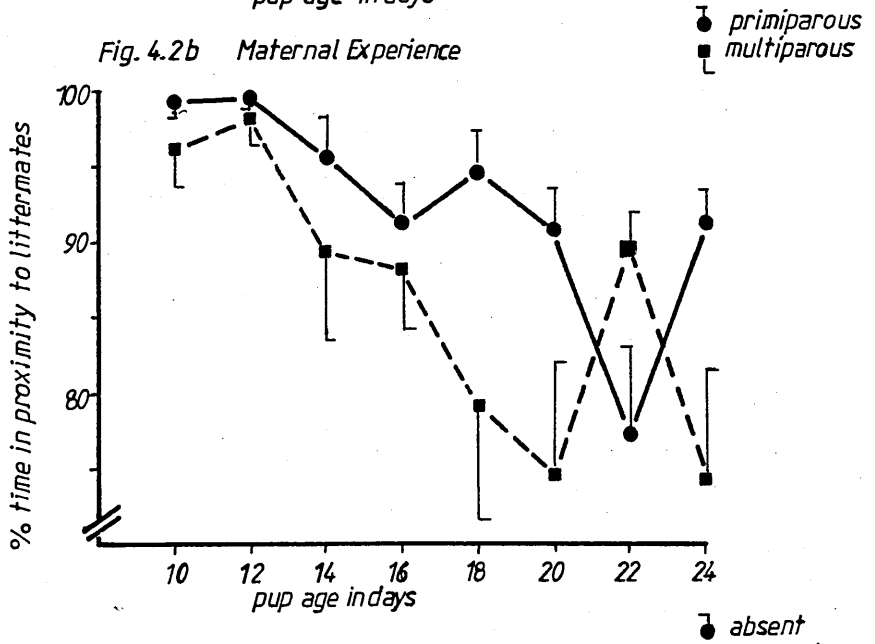
(Based on transformed data, with age as a covariate)

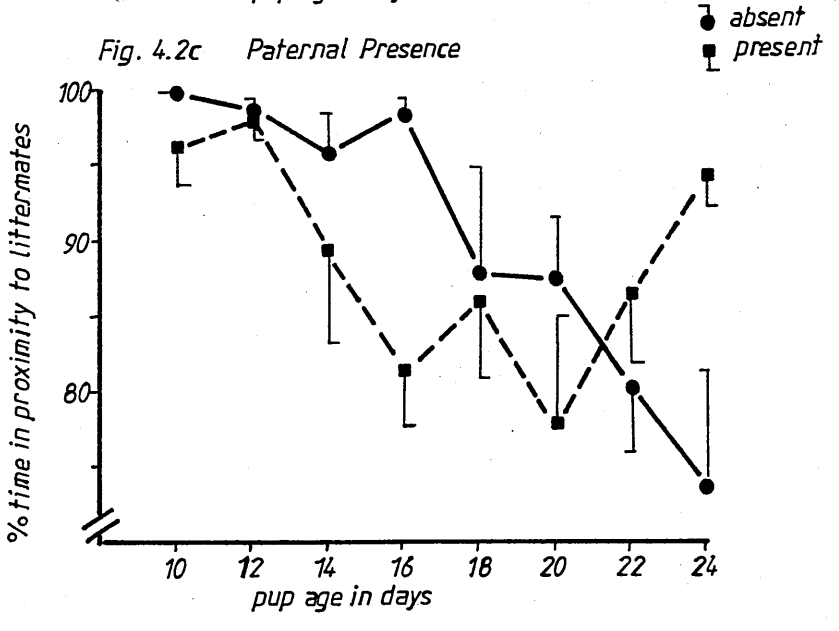
SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.992	3.329	NS
Maternal Experience (M)	1	0.449	1.507	NS
Paternal Presence (P)	1	0.133	1.945	NS
2-WAY INTERACTIONS				
L x M	1	0.298	4.352	< 0.05
L x P	1	0.031	0.450	NS
M x P	1	0.086	1.255	NS
3-WAY INTERACTION				
L x M x P	1	0.194	2.840	NS
RESIDUAL	247	0.068		

L x M

Combinations:	2-Mu	2-Pr	7-Mu	7-Pr
Means:	1.211	1.363	1.404	1.419
	(87.60)	(95.74)	(97.24)	(97.71)







Mean percentage of instantaneous scans of pup in proximity to littermates. Standard errors are indicated.

during this time, laboratory mouse pups frequently sniffed other animals, especially the father. The same workers also noted that drinking water and eating solid food were two other behaviours which characterized this stage of mouse pup development. Likely candidates, therefore, for a common focus of interest for the littermates would be the food container, the water bottle or the father.

Williams and Scott (1953) found that by day 21, mouse pups did not generally tend to aggregate as they had done at a younger pup age. There is likely to be a minimum proximity to littermate score due to the probability of one member of the litter meeting another member whilst randomly moving in a finite cage space.

3. Proximity To Mother

The ANOVA results are shown in Table 4.3, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.3a, 4.3b, and 4.3c. No main effects or interactions were significant for this activity. Proximity to mother scores were not, therefore, significantly influenced by the litter size, the maternal experience or the paternal presence factors.

Trends Associated With Increasing Age of Pups

The analysis of covariance revealed that there was no significant age effect for this measure, and the correlation analyses showed that the only treatment conditions which resulted in significant positive correlations ($P < 0.05$) were the 'small-litter' and the 'multiparous mother' ones (Table 4.A). Significant correlations between this measure and age, therefore, depend upon the treatment. The two significant correlations (Table 4.A) may be explained by a maternal litter-aversion theory.

Fig. 4.3a shows that the small-litter group had low scores at the beginning of the recording period and that these increased with pup age. The low scores at the beginning are possibly the result of a lesser suckling demand in the small litters compared to the large litters. From day 14 onwards (Fig. 4.3a), the mothers of small litters may have shown less aversion to their offspring than mothers of large litters. Seitz (1958) found that the quantity of maternal behaviour, displayed in the rat, varied inversely with the size of the litter. The positive correlation with age can, therefore, be explained by mothers of small litters allowing pups to stay close to them.

A significant age correlation is also found with pups raised by multiparous mothers (Fig. 4.3b). In common with Seitz (1958), this may be because litter aversion in the multiparous mothers had been neutralized by their greater maternal maturity and experience, compared to primiparous mothers. Consequently the increasing avoiding action which may have been taken by the primiparous mothers would explain the absence of a significant correlation, whereas a positive significant correlation is found for the multiparous mothers which may not have displayed the avoiding action (Table 4.A).

It might be expected that with increased pup independence there would be a trend of decreasing scores for proximity to the mother. King (1963) reported that Peromyscus mothers spent less time with their pups as the pup age increased. Bateman (1957) found that the time mouse mothers spent on the nest decreased from day 0 to day 15. In my own results, however, it seems that during the period when pups were spending decreasing proportions of time in the nest and in the proximity of their littermates, they were not experiencing any decrease in the proportion of time spent in close proximity to the mother.

Table 4.3

Analysis of Variance of 'Proximity To Mother' Scores

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.349	1.755	NS
Maternal Experience (M)	1	0.026	0.130	NS
Paternal Presence (P)	1	0.011	0.057	NS
2-WAY INTERACTIONS				
L x M	1	0.113	0.570	NS
L x P	1	0.002	0.008	NS
M x P	1	0.176	0.886	NS
3-WAY INTERACTION				
L x M x P	1	0.559	2.811	NS
RESIDUAL	247	0.199		

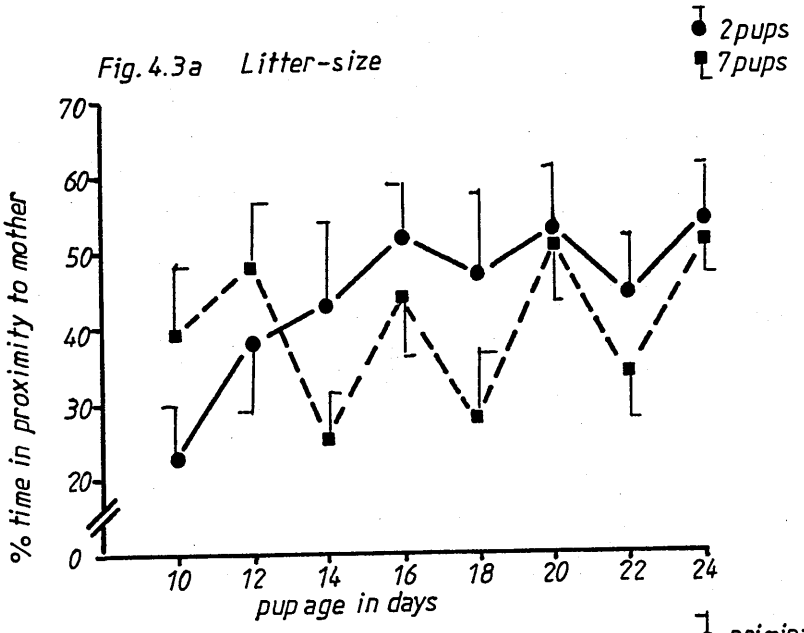


Fig. 4.3a Litter-size

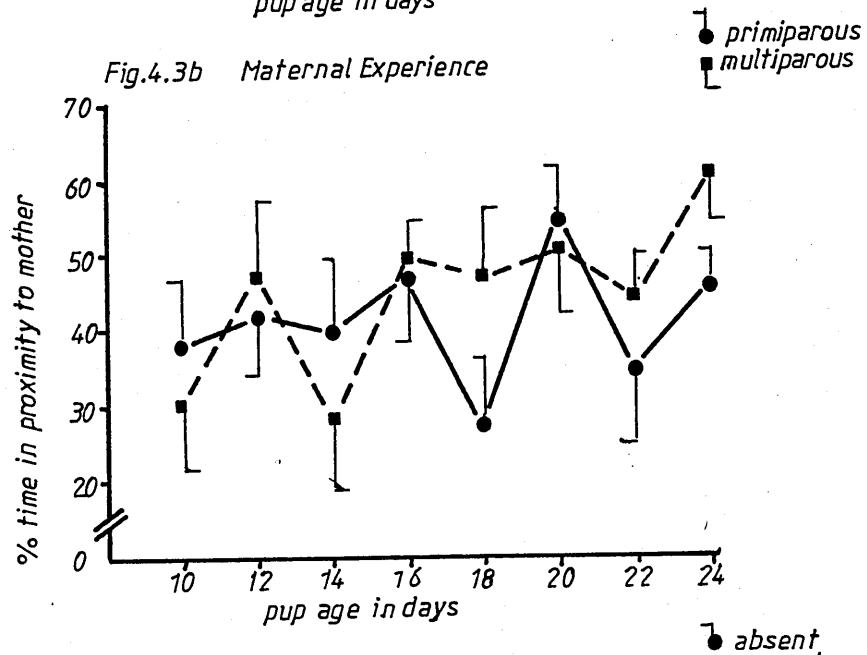


Fig.4.3b Maternal Experience

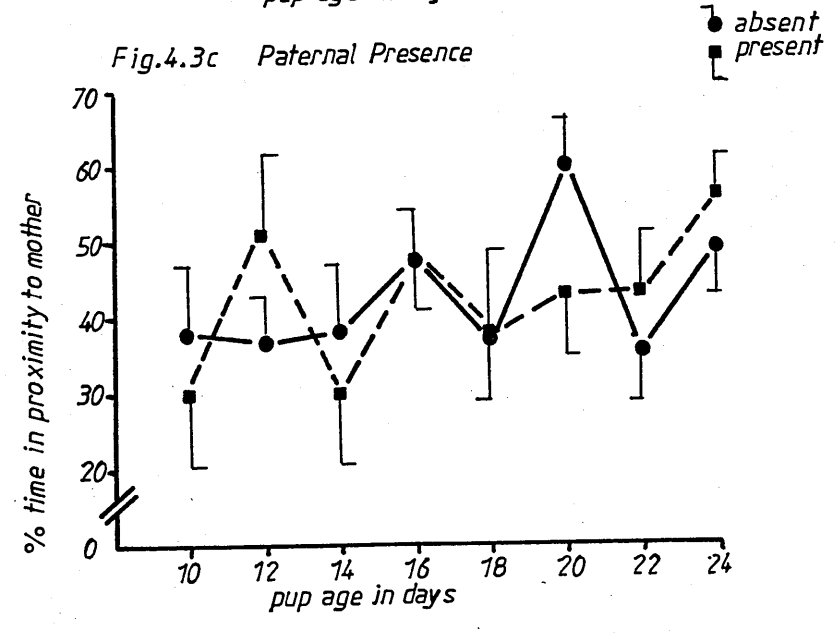


Fig.4.3c Paternal Presence

Mean percentage of instantaneous scans of pup in proximity to mother. Standard errors are indicated.

4. Proximity To Father

The ANOVA and Scheffé test results are shown in Table 4.4, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.4a and 4.4b. This is only a two-way analysis, rather than a three-way analysis in common with the others, because it was not possible to take 'proximity to father' scores in the absence of the father. Two significant main effects were apparent and these were for litter size and for maternal experience. There were no significant interactions.

Regardless of the mother's experience, litter size influenced the 'proximity to father' scores. The Scheffé test showed that overall, a significantly larger mean score was obtained for pups in small litters than for pups in large litters. This difference appeared from day 16 onwards (Figs. 4.4a). Regardless of the litter size, 'proximity to father' scores were affected by maternal experience. Pups with multiparous mothers had a significantly lower mean score than did pups with primiparous mothers. This effect was apparent during the earlier part of the recording period, up to day 21 (Fig. 4.4b).

The pups proximity to the father was influenced by the treatment effects to a far greater extent than was the pups' proximity to the mother. Paternal presence was chosen as one of the three social factors for this investigation because of the likelihood that the father would influence pup developmental behaviour (Chapter 1). This is now seen to be highly likely on the grounds that the pups spent a large proportion of time in close proximity to the father.

Trends Associated With Increasing Age of Pups

Figs. 4.4a and 4.4b indicate that, as is the case with the pups'

Table 4.4

Analysis of Variance of 'Proximity To Father' Scores with Scheffé Test Results

(Based on transformed data, with age as a covariate)

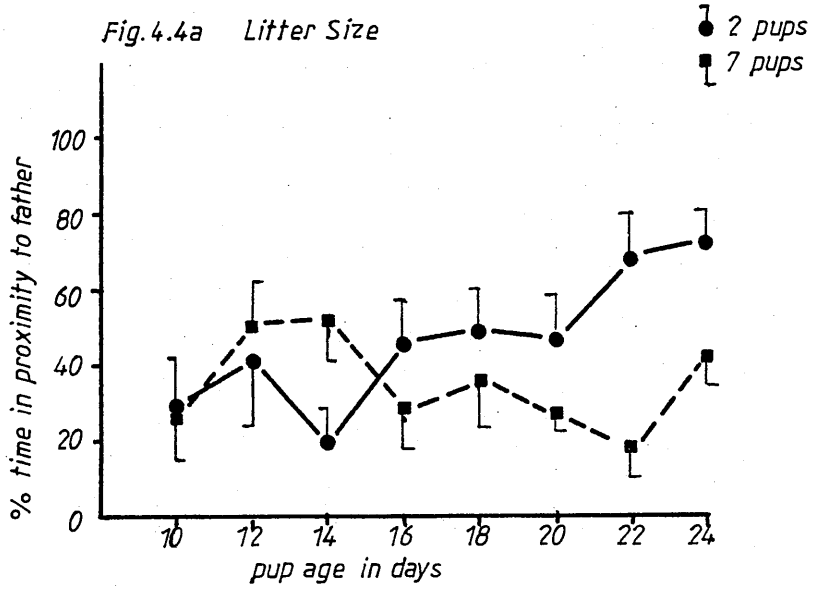
SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.801	4.157	<0.05
Maternal Experience (M)	1	1.322	6.858	<0.01
2-WAY INTERACTION				
L x M	1	0.014	0.074	NS
RESIDUAL	123	0.193		

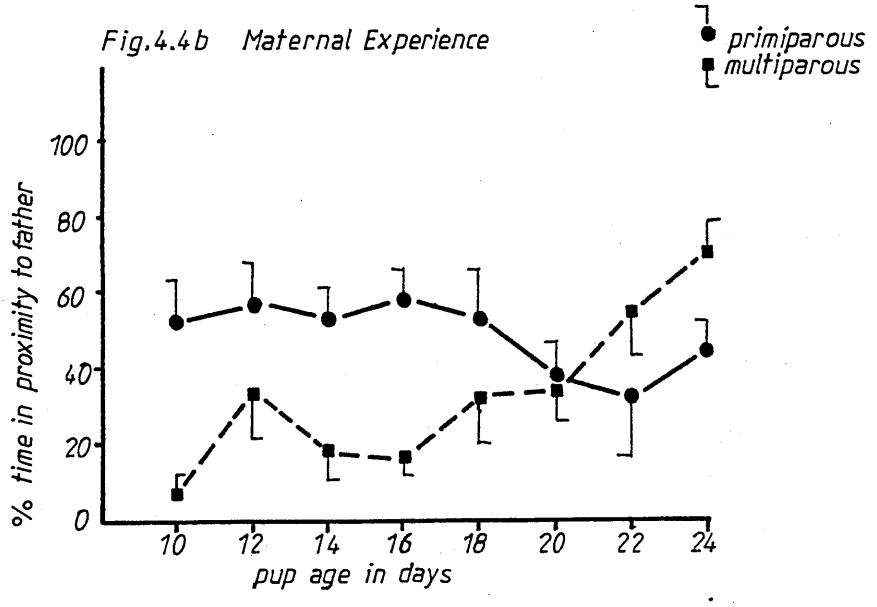
L

Litter Size:	7	2
Means:	0.590	0.748
	(30.95)	(46.26)

M

Maternal Experience	Mu	Pr
Means:	0.567	0.771
	(28.85)	(48.56)





Mean percentage of instantaneous scans of pup in proximity to father. Standard errors are indicated.

proximity to the mother, pups continued to spend a high proportion of time in proximity to the father throughout the recording period. The analysis of covariance revealed that there was no significant age effect for this measure, but the correlation analyses showed that for the small-litter group and for the 'multiparous mother' group, there was a significant positive correlation ($P < 0.001$) between this measure and age (Table 4.A). It should be noticed that the pattern of significant and non significant correlations is the same as that for the 'proximity to mother' analysis (Table 4.A). It is possible that the fathers of large litters displayed an increasing aversion to their offspring as the pup age increased. Older pups would constitute a greater caretaking load than younger pups. The small litters did not appear to evoke the same reaction in the fathers, presumably because they represented a reduced caretaking load, compared to the large litters. This would explain the positive correlation in the small-litter scores (Fig. 4.4a).

Although the 'multiparous mother' pups spent, overall, less time in close proximity to the father compared to the 'primiparous mother' pups, the scores for the former group do show an increase with pup age which is not shown in the scores obtained from the latter group (Fig. 4.4b). This positive correlation with age found with the 'multiparous mother' group may have been caused by a willingness on the part of the fathers to spend increasing time in close proximity to the pups as the pups became older. Since the father's caretaking experience corresponded with that of the mothers', this result may reflect a greater degree of aversion to older pups in the less experienced fathers. This is the same explanation as that advanced to explain the similar pattern of results for the 'proximity to mother' measure.

5. Locomotor Behaviour

The ANOVA results are shown in Table 4.5 and the graphs of the means for each main factor averaged over the other main factors, are shown in Figs. 4.5a, 4.5b and 4.5c. No main effects or interactions were significant for this activity. 'Locomotor behaviour' scores, therefore, were not significantly influenced by any of the three independent variables.

Trends Associated With Increasing Age of Pups

The analysis of covariance revealed a significant age effect ($P < 0.01$) and the correlation analyses showed a significant negative correlation ($P < 0.05$) for the 'large-litter', 'multiparous mother' and 'father absent' groups (Table 4.A). Figs. 4.5a, 4.5b and 4.5c also show a slight trend of decreasing scores with pup age.

It is likely that the disruptions on days 14/15, which are clearly out of step with the general age trend, contributed to the low correlations obtained for this behaviour (Table 4.A). Days 14/15 coincide with the age when pups first begin to leave their nest to a greater extent and when extremely intense and undirected flurries of activity are commonly reported (Williams & Scott 1953).

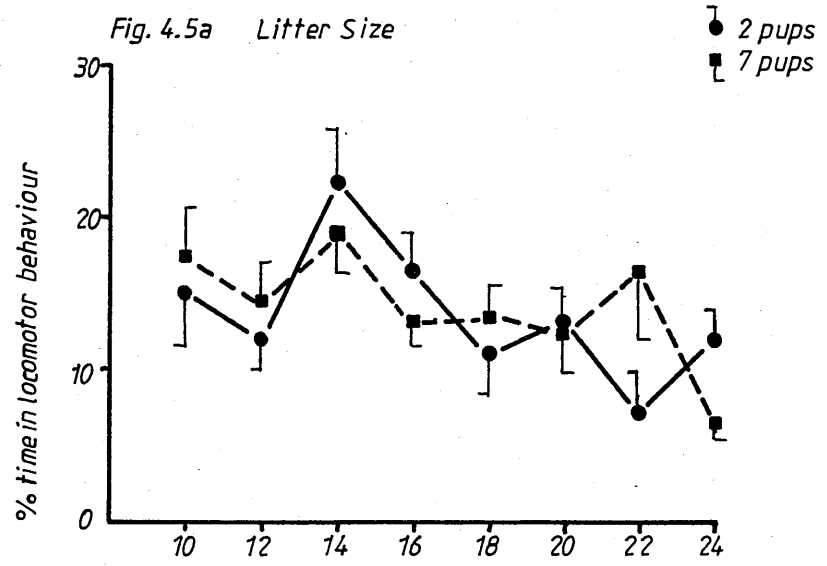
It should be appreciated that 'locomotor behaviour' is a blanket term covering a wide range of different activities. It was observed that predominant locomotor activities in the first few days of life were crawling and kicking. Later, standing and walking became possible, and eventually those activities typical of adult locomotion appeared. Such activities included jumping, running, digging and climbing. During the pre-weaning period some of the early locomotor activities would diminish with age as other locomotor activities would increase. The locomotor

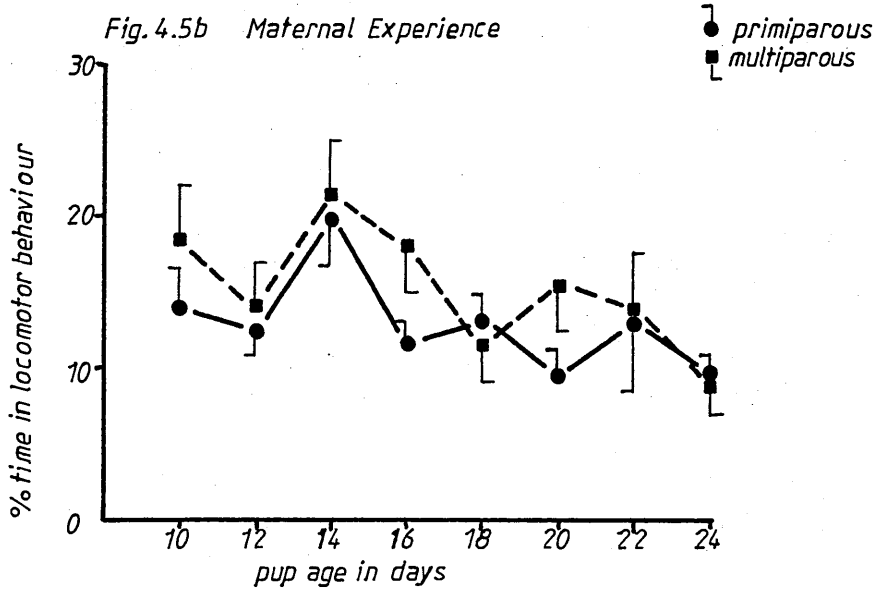
Table 4.5

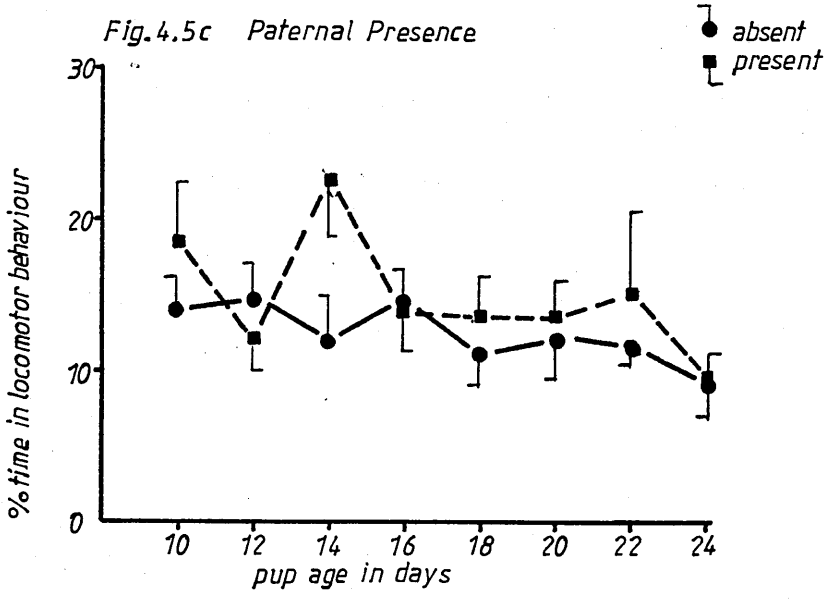
Analysis of Variance of 'Locomotor Behaviour' Scores

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.001	0.049	NS
Maternal Experience (M)	1	0.031	1.031	NS
Paternal Presence (P)	1	0.015	0.499	NS
2-WAY INTERACTIONS				
L x M	1	0.017	0.570	NS
L x P	1	0.038	1.257	NS
M x P	1	0.004	0.134	NS
3-WAY INTERACTION				
L x M x P	1	0.073	2.411	NS
RESIDUAL	247	0.030		







Mean percentage of instantaneous scans of pup in locomotor behaviour. Standard errors are indicated.

behaviour scores do not monitor the development and diminution of specific and individual locomotor activities therefore, but the extent to which any type of locomotor behaviour is being displayed.

Some of the 12 activities which were recorded on a daily basis are mutually exclusive. It is unlikely that rearing, grooming, eating solid food and drinking would occur at the same time as the subject was displaying locomotor behaviour. These four activities, which compete for time with locomotor behaviour, were displayed more frequently towards the end of the recording period. During the earlier part of pre-weaning development, when many non-locomotor activities were not yet part of the subject's behavioural repertoire, it is conceivable that more time would be spent displaying locomotor behaviour. This may provide an explanation for the downward trend of locomotor behaviour with pup age.

Locomotor behaviour scores decreasing with age are not, therefore, necessarily indicative of a trend towards lower activity, but may instead be reflecting the increase of other non-locomotor activities. It is not the intention of this work to examine the course of development of each of the different types of locomotion. Williams & Scott (1953) provide additional information on locomotor development in mice and Altman & Sudarshan (1975) provide useful and detailed information on locomotor development in rats. Much of this latter work is based, however, on the results of reflexological tests as opposed to purely observational studies.

6. Sniffing

The ANOVA and Scheffé test results are shown in Table 4.6, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.6a, 4.6b and 4.6c. A significant three way interaction exists involving litter size, maternal experience and paternal presence. The Scheffé test revealed that there was a significant difference between the 7-Mu-M mean and the 2-Pr-F mean. The former combination resulted in a lower mean score than the latter.

Trends Associated With Increasing Age of Pups

The analysis of covariance showed a significant age effect ($P < 0.001$) for this measure and the correlation analyses showed that in all treatment conditions except for that of 'father present', a significant positive correlation between this activity and age was apparent (Table 4.A). Fig. 4.6c shows that the 'father present' scores do not exhibit the same gradual increase with age as the other treatment scores. One contributory reason for this is the already high scores towards the beginning of the recording period. Increased sniffing scores at this time are likely to represent the pup's response to the father's presence. It seems likely that the pups are able to detect the father's odours. This would explain the absence of a positive correlation with age in the 'father present' scores (Table 4.A).

Sniffing was the first of the three exploratory activities to be observed in the infant mice. By day 10, which was the first day of the recording period, sniffing was observed. There were two major spurts in this activity. One was between days 12/13 and 14/15, and the other was between days 18/19 and 20/21 (Figs. 4.6a, 4.6b and 4.6c).

Table 4.6

Analysis of Variance of 'Sniffing' Scores
with Scheffé Test Results

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.055	0.195	NS
Maternal Experience (M)	1	0.000	0.000	NS
Paternal Presence (P)	1	0.184	0.652	NS
2-WAY INTERACTIONS				
L x M	1	0.000	0.000	NS
L x P	1	0.007	0.025	NS
M x P	1	0.004	0.014	NS
3-WAY INTERACTION				
L x M x P	1	0.282	11.168	=0.001
RESIDUAL	247	0.025		

L x M x P

Combinations:	7-Mu-M	2-Pr-M	7-Pr-F	7-Pr-M	2-Mu-F	2-Mu-M	7-Mu-F	2-Pr-F
Means:	0.137	0.187	0.205	0.215	0.230	0.246	0.276	0.288
	(1.86)	(3.46)	(4.14)	(4.55)	(5.20)	(5.93)	(7.43)	(8.07)

Fig.4.6a Litter Size

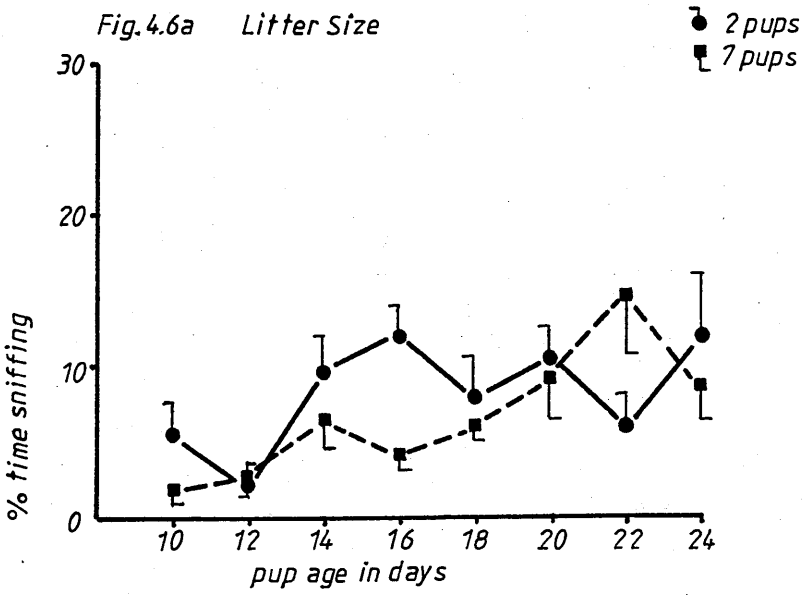


Fig.4.6b Maternal Experience

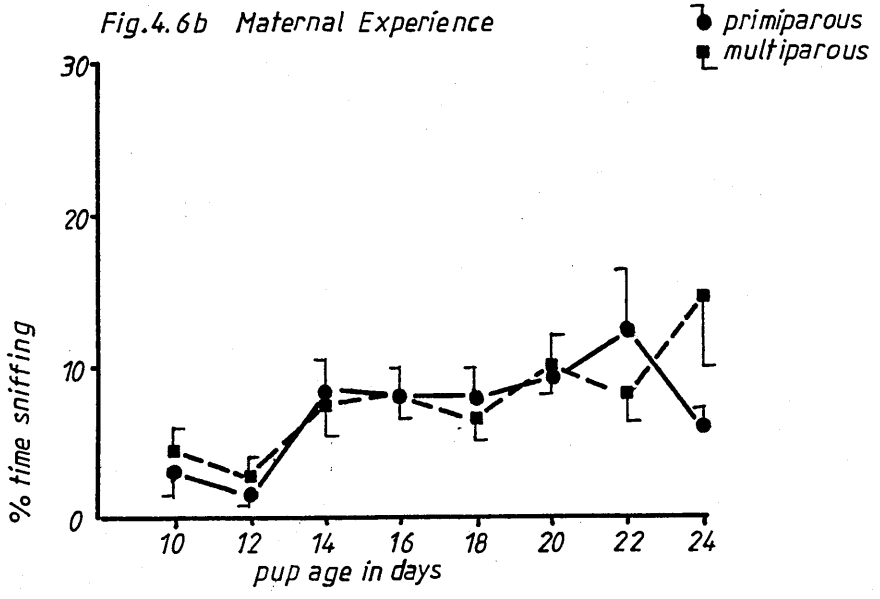
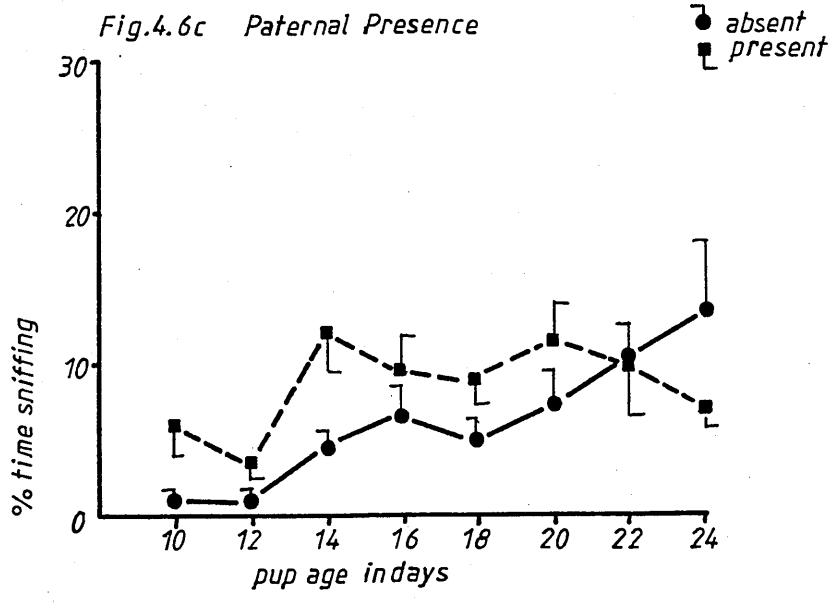


Fig.4.6c Paternal Presence



Mean percentage of instantaneous scans of pup sniffing.
Standard errors are indicated.

7. Head-Lifting

The ANOVA and Scheffé test results are shown in Table 4.7, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.7a, 4.7b and 4.7c. Two significant main effect differences were found with the head-lifting analysis. Litter-size was one of these and the Scheffé test showed that the small litter mean was significantly larger than the large-litter mean. A pup from a small litter, therefore, tended to head-lift for a greater proportion of time than a pup from a large litter.

The other significant main effect was that of paternal presence. A significantly higher mean was obtained with those pups reared in the presence of the father, compared to those reared without the father.

Trends Associated With Increasing Age of Pups

The analysis of covariance showed a significant age effect ($P < 0.05$) for this measure and the correlation analyses revealed that there were significant positive correlations between age and this measure for the large-litter and the 'father absent' groups (Table 4.A). A study of the graph shapes is useful whilst interpreting these results. Fig. 4.7a shows the difference in graph shape for the two litter-size groups. The small-litter mean scores are generally high, but decrease at the end of the recording period whereas the large-litter mean scores are generally lower throughout, but do nevertheless show a gradual increase. It is this increase which is reflected in the correlation result. It is possible that this observed difference is due to delayed development of the large-litter pups, in comparison with the small-litter pups. Fig. 4.7c shows the difference in graph shape for the two 'paternal presence' groups. A similar pattern is observed here as in

the litter-size comparison. The 'father present' scores are generally high, but decrease towards the end of the recording period whereas the 'father absent' scores are generally lower throughout, but show a gradual increase throughout most of the recording period. It is, once again, this increase which results in the significant correlation for the 'father absent' scores. There may again be similar trends in the two groups, but with the 'father absent' pups showing delayed development in comparison with the 'father present' pups.

In common with sniffing, head-lifting was observed from the beginning of the recording period (Figs. 4.7a, 4.7b and 4.7c). Also in common with sniffing, increases in scores were apparent between days 12/13 and 14/15 and between days 18/19 and 20/21. These two increases may signify particular developmental stages. The first one coincided with the time pups began to leave the nest. Towards the end of the recording period, the scores entered a phase of decline. Williams & Scott (1953) found that developing mice were increasingly investigating the cage and other animals, from day 12 onwards.

Table 4.7

Analysis of Variance of 'Head-Lifting' Scores
with Scheffé Test Results

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.039	4.441	<0.05
Maternal Experience (M)	1	0.002	0.177	NS
Paternal Presence (P)	1	0.082	9.391	<0.01
2-WAY INTERACTIONS				
L x M	1	0.016	1.861	NS
L x P	1	0.001	0.078	NS
M x P	1	0.001	0.114	NS
3-WAY INTERACTION				
L x M x P	1	0.003	0.339	NS
RESIDUAL	247	0.009		

L

Litter Size:	7	2
Means:	0.079	0.104
	(0.62)	(1.08)

P

Paternal Presence:	M	F
Means:	0.073	0.109
	(0.53)	(1.18)

Fig.4.7a Litter Size

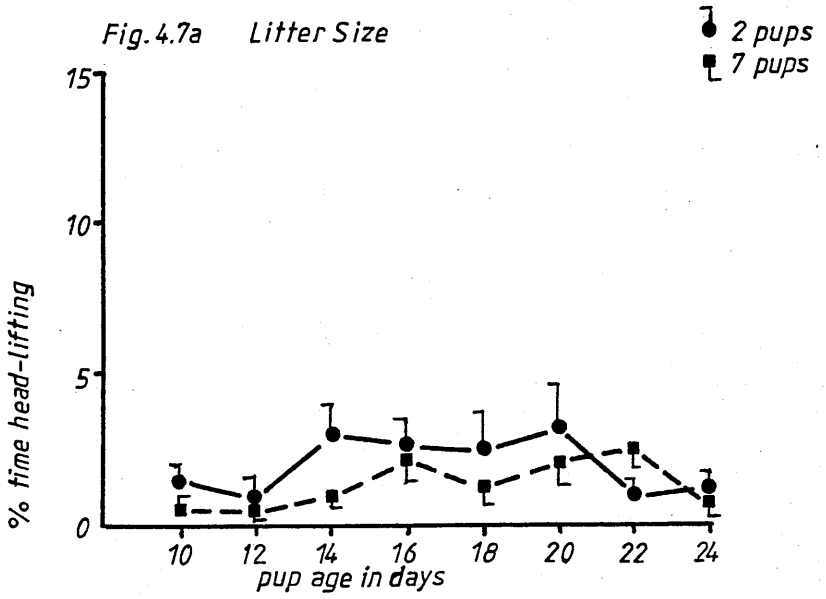


Fig.4.7b Maternal Experience

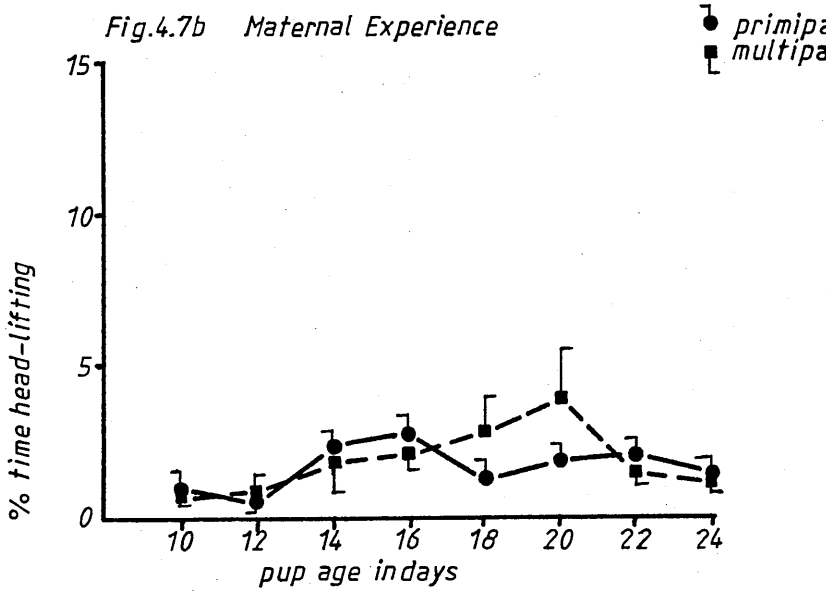
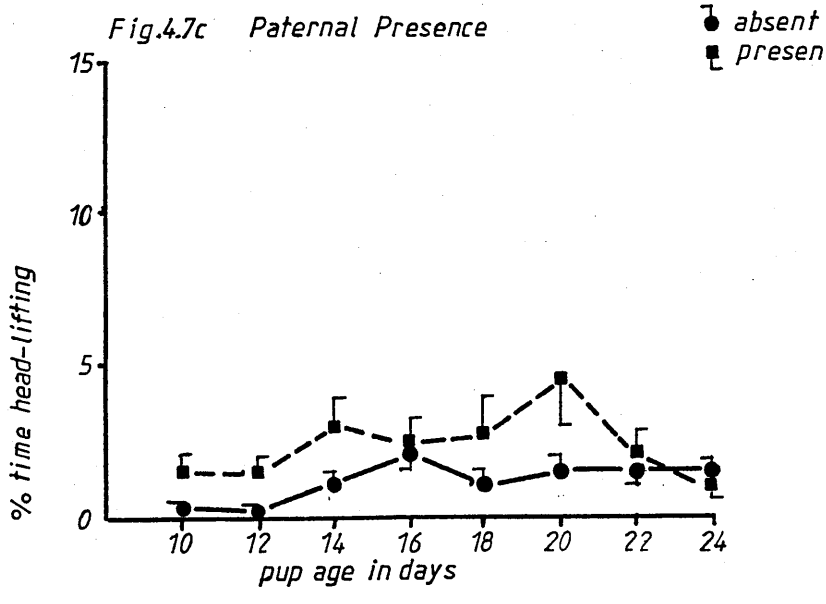


Fig.4.7c Paternal Presence



Mean percentage of instantaneous scans of pup head-lifting.
Standard errors are indicated.

8. Rearing

The ANOVA and Scheffé test results are shown in Table 4.8, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.8a, 4.8b and 4.8c. The ANOVA table shows that there is a significant difference for one main effect and another significant difference for a two-way interaction.

The main effect concerns 'paternal presence' and the Scheffé test revealed that the mean, obtained from pups raised in the presence of the father, is significantly higher than the mean obtained from pups raised without the father.

No significant differences were apparent between means of combinations of factors in the L x M two-way interaction using the Scheffé test. The greatest difference between the means, however, was found between the 2-Pr subjects and the 2-Mu subjects, where the latter group obtained the highest mean value.

Trends Associated With Increasing Age of Pups

The analysis of covariance showed a significant age trend ($P < 0.001$) for this measure and the correlation analyses revealed that there were significant positive correlations between age and this measure for the large-litter, the 'primiparous mother' and the 'father absent' groups (Table 4.A). An examination of Figs. 4.8a, 4.8b and 4.8c reveals that these rearing trends with age show a similar pattern to those observed in head-lifting. The three treatments exhibiting a significant positive correlation with age may actually be associated with delayed development in comparison with the three treatments showing no significant correlation with age. It should be noticed

Table 4.8

Analysis of Variance of 'Rearing' Scores
with Scheffé Test Results

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.001	0.063	NS
Maternal Experience (M)	1	0.005	0.383	NS
Paternal Presence (P)	1	0.078	5.975	<0.05
2-WAY INTERACTIONS				
L x M	1	0.085	6.466	<0.05
L x P	1	0.001	0.050	NS
M x P	1	0.022	1.667	NS
3-WAY INTERACTION				
L x M x P	1	0.003	0.195	NS
RESIDUAL	247	0.013		

P

Paternal Presence:	M	F
Means:	0.051	0.086
	(0.26)	(0.74)

L x M

Combinations:	2-Pr	7-Mu	7-Pr	2-Mu
Means:	0.048	0.053	0.081	0.093
	(0.23)	(0.28)	(0.65)	(0.86)

Fig.4.8a Litter Size

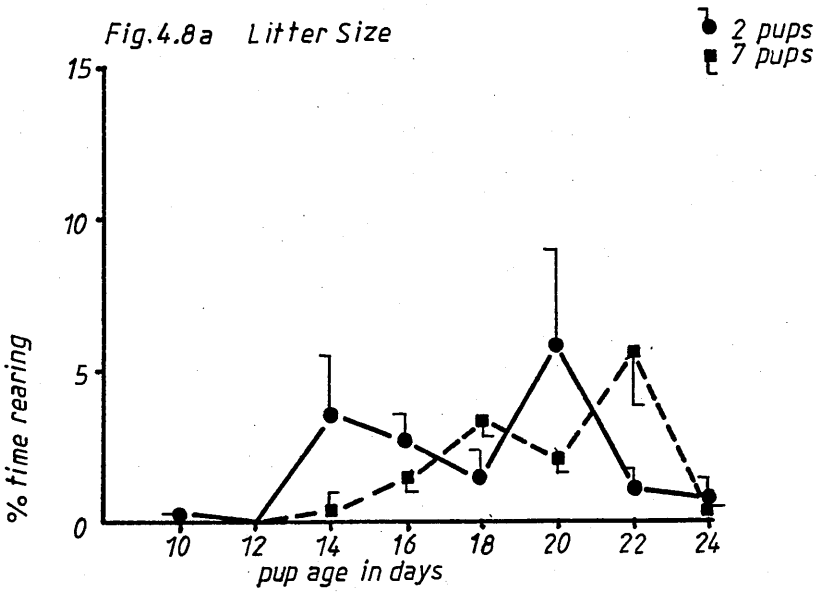


Fig.4.8b Maternal Experience

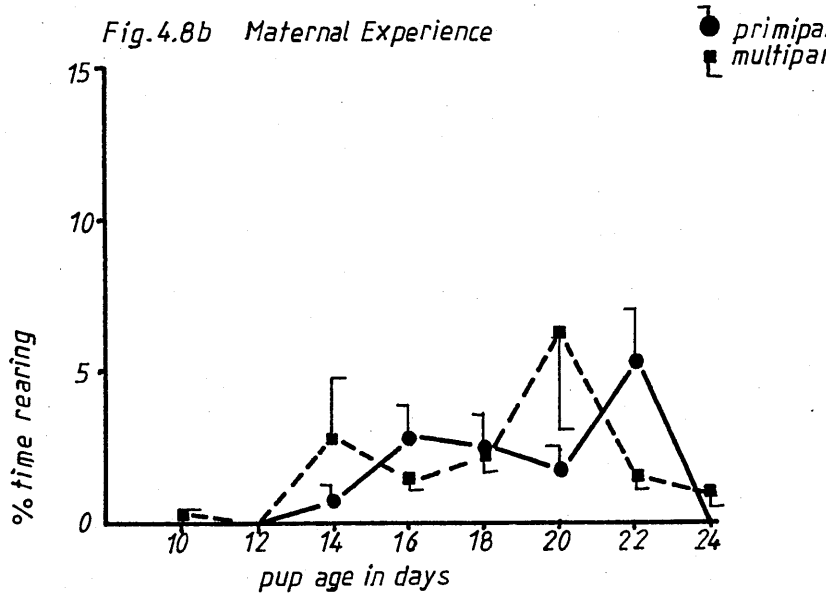
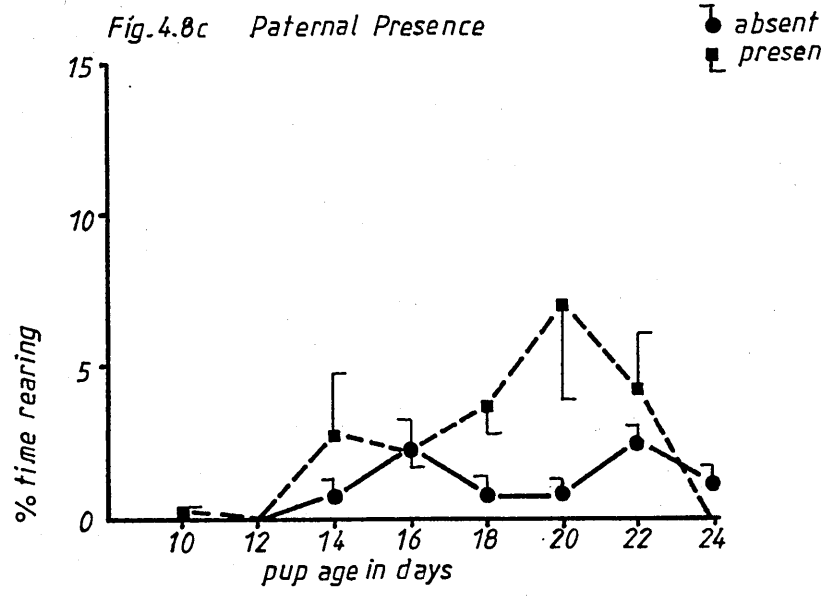


Fig.4.8c Paternal Presence



Mean percentage of instantaneous scans of pup rearing.
Standard errors are indicated.

that this latter group have scores which enter a decline phase at an earlier age than the former group. The incidence of rearing may provide a developmental rate index. If this is the case, then the small litter size, the multiparous mother and the father's presence are all associated with faster development in mouse pups.

Rearing was not observed to any great extent until day 14, although it has been suggested that it occurs from day 12 (Williams & Scott 1953). There was, in common with the other two exploratory activities, an increase in this activity between days 12/13 and 14/15 (Figs. 4.8a, 4.8b and 4.8c). Such a finding may indicate that the three exploratory activities are related to each other in terms of when they are performed. In a similar fashion to the head-lifting scores, the rearing scores entered a decline phase before the end of the recording period. It would be interesting to know future trends with pup age past day 25.

Care must be exercised when considering the rearing scores since rearing may be a multiple-function activity. Altman & Sudarshan (1975) acknowledged its role as an acute form of exploratory behaviour, but also pointed out its function as a preliminary to climbing. Rearing would also be required when obtaining food from the cage-lid dispenser. It cannot, therefore, be assumed that high rearing scores totally represent increased exploratory behaviour.

9. Grooming

The ANOVA and Scheffé test results are shown in Table 4.9 and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.9a, 4.9b and 4.9c. There is a significant three-way interaction involving litter size, maternal experience and paternal presence. The Scheffé test identified two significant differences between means of particular combinations of factors. The 7-Mu-M mean was significantly smaller than both the 2-Pr-F mean and the 7-Mu-F mean.

Trends Associated With Increasing Age of Pups

The analysis of covariance showed a significant age trend ($P < 0.001$) for this measure and the correlation analyses revealed that there were significant positive correlations ($P < 0.001$) between age and this measure for all treatment groups (Table 4.A). This clear age trend is shown in Figs. 4.9a, 4.9b and 4.9c. It is possible than an increase in self-grooming by the pups, which depended upon the development of their motor skills, was accompanied by a decline in the amount of grooming, pups received from their parents. One exception to the trend of increasing scores with pup age was the result obtained on days 22/23, when scores were markedly depressed.

Grooming behaviour in the early post-natal period involved rudimentary actions of the type exhibited in the late pre-weaning period. Ineffectual attempts at hindlimb and forelimb scratching of the nose and ear could be observed first; and then later, licking of the forepaws, anogenital region, tail and body fur occurred. Ultimately grooming

Table 4.9

Analysis of Variance of 'Grooming' Scores
with Scheffé Test Results

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.014	0.035	NS
Maternal Experience (M)	1	0.060	0.149	NS
Paternal Presence (P)	1	0.407	1.010	NS
2-WAY INTERACTIONS				
L x M	1	0.000	0.000	NS
L x P	1	0.112	0.278	NS
M x P	1	0.354	0.878	NS
3-WAY INTERACTION				
L x M x P	1	0.403	9.653	<0.01
RESIDUAL	247	0.042		

L x M x P

Combinations:	7-Mu-M	2-Mu-M	7-Pr-F	2-Pr-M	2-Mu-F	7-Pr-M	2-Pr-F	7-Mu-F
Means:	0.171	0.305	0.321	0.332	0.338	0.353	0.375	0.446
	(2.90)	(9.02)	(9.95)	(10.62)	(11.00)	(11.95)	(13.42)	(18.61)

Fig.4.9a Litter Size

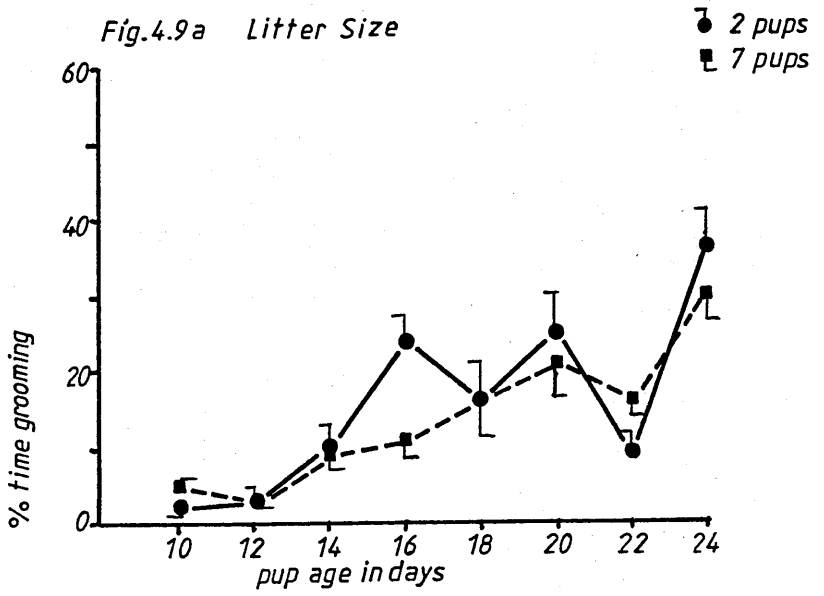


Fig.4.9b Maternal Experience

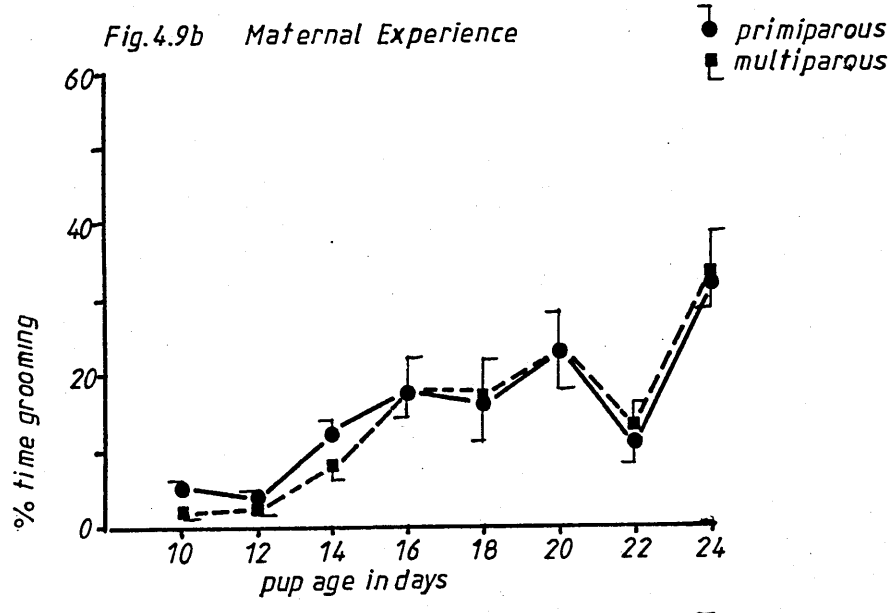
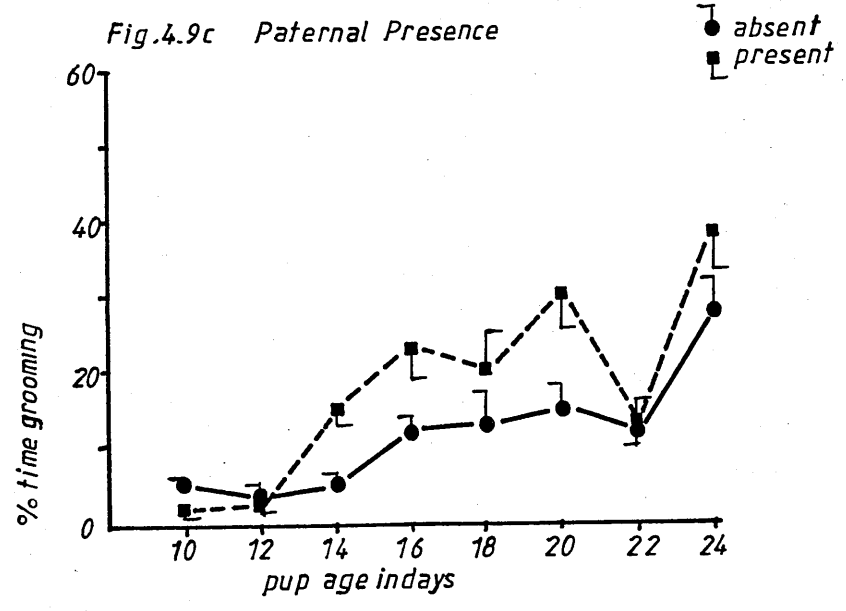


Fig.4.9c Paternal Presence



Mean percentage of instantaneous scans of pup grooming.
Standard errors are indicated.

was characterized by intensive cleaning of the face by the forepaws, and by hindlimb scratching of the body. Since these grooming actions presuppose some functional maturation of the brain, and the fore and hindlimbs, the incidence of certain types of grooming could be used to measure degrees of physical development. Fentress (1978) examined the ontogeny of grooming in the laboratory mouse and concluded that grooming development was characterized by three stages of increasing muscular control and coordination. Williams & Scott (1953) described the development of mouse grooming from the occasional initial weak attempts at grooming, to the later displays of fully-developed and effective grooming patterns. The grooming of littermates by the subjects was observed later in the pre-weaning period, but the occurrence of this was not recorded.

10. Suckling

The ANOVA and Scheffé test results are shown in Table 4.10, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.10a, 4.10b and 4.10c. There was one significant difference and this was for a main effect. The litter-size factor affected suckling scores and it was the larger size litter which had a significantly greater mean value than the small size litter.

Trends Associated With Increasing Age of Pups

The analysis of covariance showed no significant age effect for this measure, and the correlation analyses revealed that the only treatment conditions which have a significant negative correlation with age ($P < 0.05$) are the 'primiparous mother' and 'father present' ones (Table 4.A). The low correlation values indicate the absence of an expected clear decline with age for this activity. Suckling was continued throughout the entire recording period, through to an age of 25 days. Nevertheless the 'primiparous mother' and 'father present' scores show a general decrease with pup age. Fig. 4.10b shows that the two 'maternal experience' trends are similar except that the 'primiparous mother' scores go into an earlier decline (day 16 onwards). A decline in suckling is likely to be due to either decreased suckling demand by the pups or decreased proclivity to nurse by the mothers. The latter reason may be pertinent here since it is likely to be the less experienced mothers which develop a greater aversion to the growing and increasingly demanding offspring (Seitz 1958).

For the 'father present' trend, Fig. 4.10c reveals two areas of interest. A high score on days 12/13 is dependent on the mother being

present in the nest to provide the suckling opportunity. It is apparent, therefore, that the father's presence is not to be equated with maternal nest aversion. The lower scores from day 16 onwards may indicate accelerated development in the 'father present' pups, in comparison with the 'father absent' pups.

Bateman (1957) found that the mean length of the nursing period decreased with pup age in mice. It should be noted, however, that at the close of the recording period in my own study, approximately 10 per cent of the observation time, on average, was still being spent by the pups suckling. It was not uncommon to observe an older pup approach the mother and begin suckling when the mother was well away from the nest area and involved in a specific activity such as eating or drinking. Whereas the mother's position, in or out of the nest, is a determinant of the amount of suckling which can take place in the early pre-weaning stages; pup initiation of suckling becomes an important factor in the onset of suckling bouts once the pups are mobile.

Table 4.10

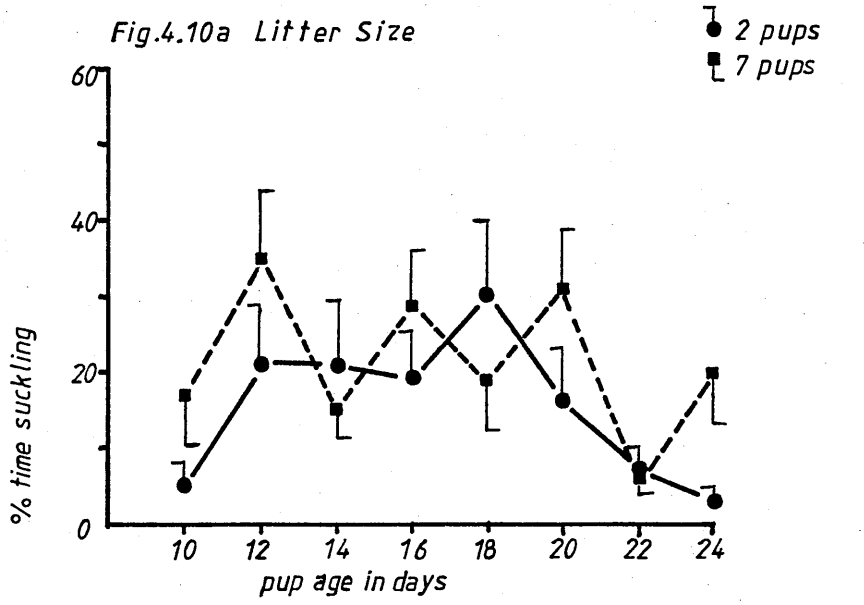
Analysis of Variance of 'Suckling' Scores
with Scheffé Test Results

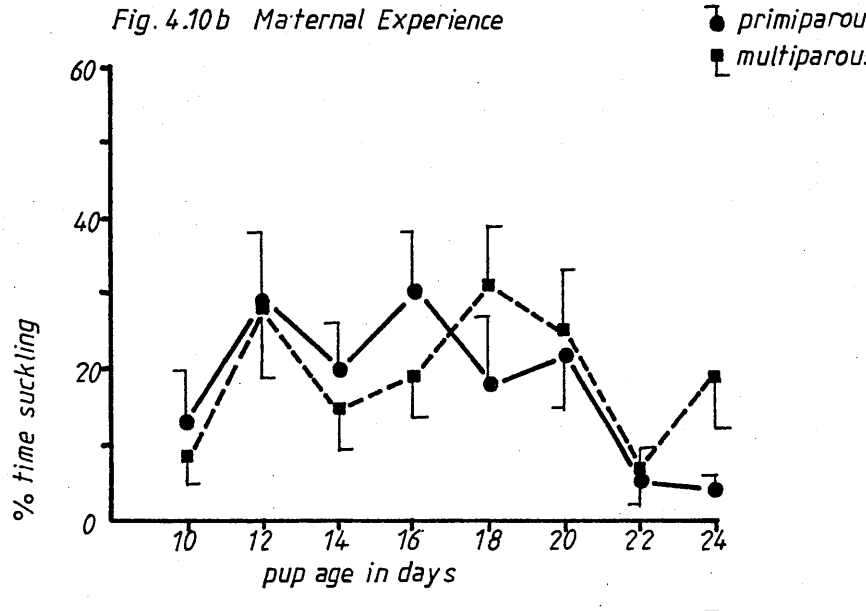
(Based on transformed data, with age as a covariate)

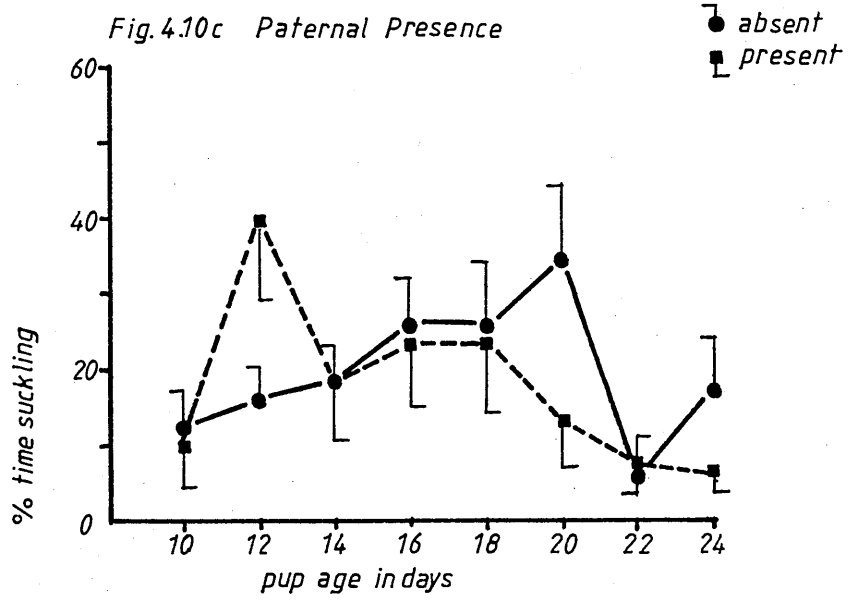
SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.896	5.489	< 0.05
Maternal Experience (M)	1	0.059	0.361	NS
Paternal Presence (P)	1	0.246	1.504	NS
2-WAY INTERACTIONS				
L x M	1	0.028	0.174	NS
L x P	1	0.000	0.003	NS
M x P	1	0.150	0.919	NS
3-WAY INTERACTION				
L x M x P	1	0.290	1.775	NS
RESIDUAL	247	0.163		

L

Litter Size:	2	7
Means:	0.283	0.402
	(7.80)	(15.31)







Mean percentage of instantaneous scans of pup suckling.
Standard errors are indicated.

11. Eating Solid Food

The ANOVA and Scheffé test results are shown in Table 4.11, and the graphs of the means for each main factor averaged over the other main factors are shown in Figs. 4.11a, 4.11b and 4.11c. There was one significant main effect; namely that of paternal presence. When the father was present with the developing pups, a significantly higher mean score was obtained than when the father was absent.

Trends Associated With Increasing Age of Pups

The analysis of covariance showed a significant age trend ($P < 0.001$) and the correlation analyses showed that significant positive correlations ($P < 0.001$) were found for all treatment conditions (Table 4.A). Figs. 4.11a, 4.11b and 4.11c show that solid food was eaten from day 14 onwards and that there was an expected increase throughout the recording period. It is worth commenting on the 'maternal experience' age trends (Fig. 4.11b) where although both showed a significant correlation ($P < 0.001$), the two trends appear to exhibit different gradients. Although casual inspection of Fig. 4.11b suggests that the extent to which feeding corresponds with age depends upon maternal experience, in fact the two correlation coefficients do not differ significantly at the 5 % level (z test for two Pearson correlation coefficients).

It is of interest that Williams & Scott (1953) record a later age of 17 days for early ingestion of food in laboratory mice. I was able to observe that fragmented pellets of food were initially selected from the cage sawdust and eaten with the aid of the forepaws. Eventually food could be obtained from the cage-lid dispenser, once the necessary physical development had taken place to allow this.

Table 4.11

Analysis of Variance of 'Eating Solid Food' Scores with Scheffé Test Results

(Based on transformed data, with age as a covariate)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	0.008	0.261	NS
Maternal Experience (M)	1	0.009	0.304	NS
Paternal Presence (P)	1	0.149	5.178	<0.05
2-WAY INTERACTIONS				
L x M	1	0.059	2.034	NS
L x P	1	0.004	0.137	NS
M x P	1	0.001	0.032	NS
3-WAY INTERACTION				
L x M x P	1	0.037	1.284	NS
RESIDUAL	247	0.029		

P

Paternal Presence:	M	F
Means:	0.108	0.156
	(1.16)	(2.41)

Fig. 4.11a Litter Size

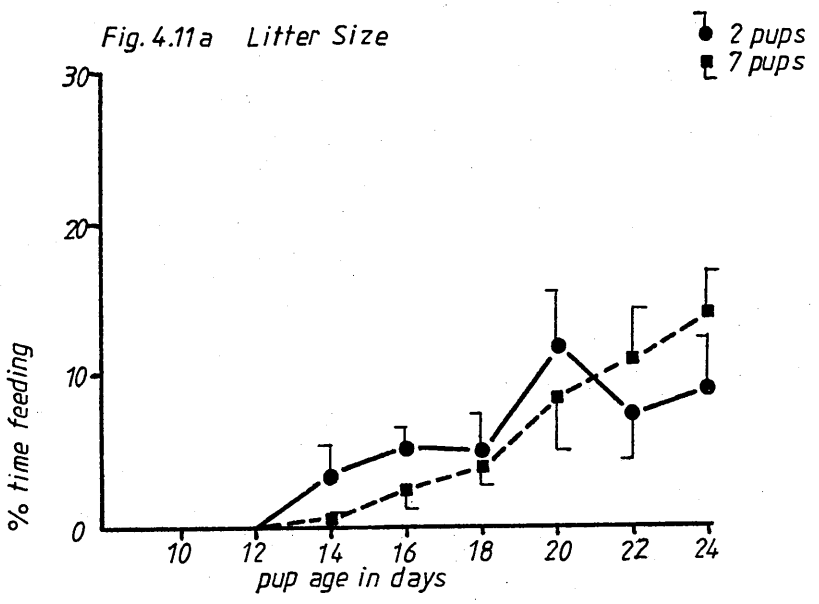


Fig. 4.11b Maternal Experience

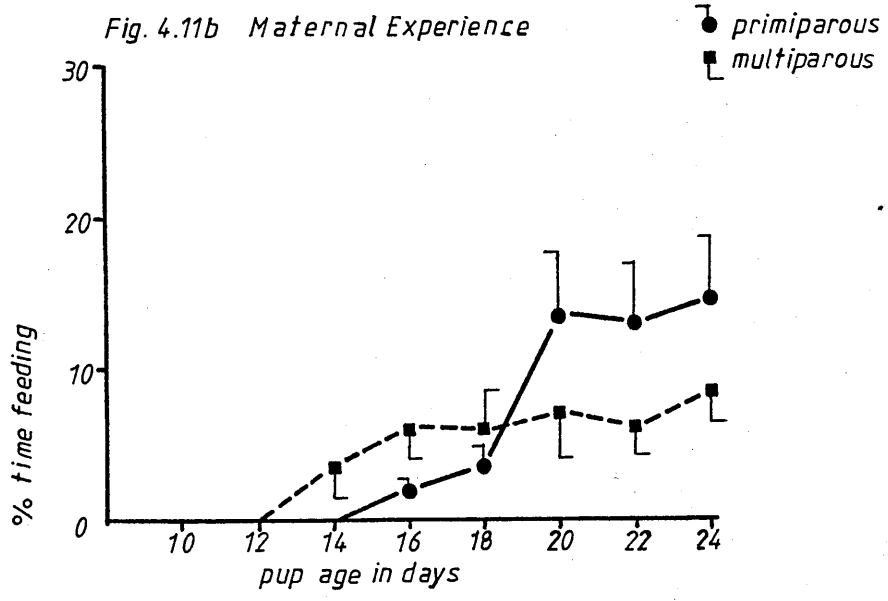
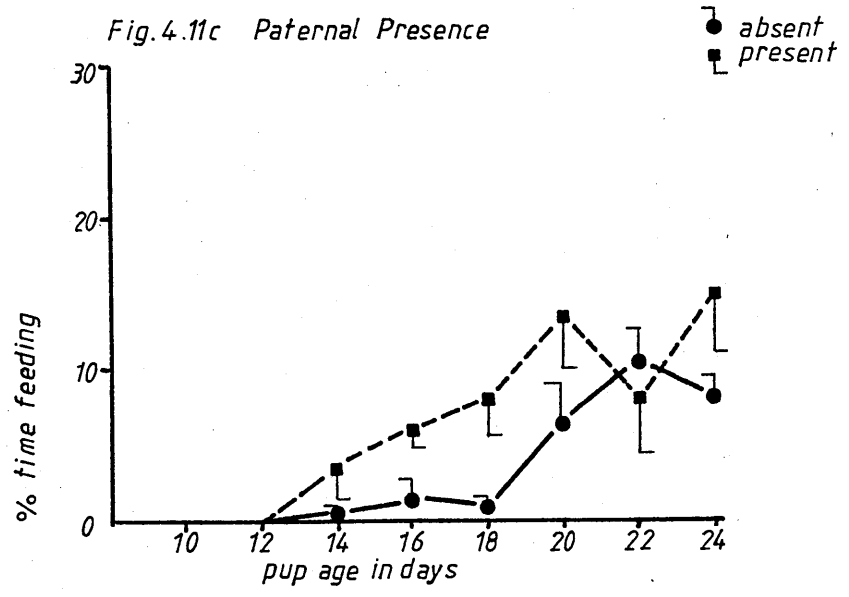


Fig. 4.11c Paternal Presence



Mean percentage of instantaneous scans of pup feeding [solid food]. Standard errors are indicated.

12. Open Field, Peripheral Compartment Ambulation

The ANOVA and Scheffé test results are shown in Table 4.12, and the histograms for the means of each main factor averaged over the other main factors are shown in Figs. 4.12a, 4.12b and 4.12c. A significant difference was found with the three-way interaction where the 2-Mu-M group had a significantly smaller mean value than the 2-Pr-M group. The varying magnitudes of the means reveal the wide-ranging scores obtained with this test.

Table 4.12

Analysis of Variance of Open Field 'Peripheral Compartment' Ambulation Scores with Scheffé Test Results

(Based on untransformed data)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	968.00	0.091	NS
Maternal Experience (M)	1	1104.50	0.104	NS
Paternal Presence (P)	1	276.12	0.026	NS
2-WAY INTERACTIONS				
L x M	1	4704.50	0.444	NS
L x P	1	120.13	0.111	NS
M x P	1	16471.13	1.556	NS
3-WAY INTERACTION				
L x M x P	1	10585.12	6.491	<0.05
RESIDUAL	24	1630.60		

L x M x P

Combinations:	2-Mu-M	2-Pr-F	7-Pr-F	7-Pr-M	7-Mu-M	7-Mu-F	2-Mu-F	2-Pr-M
Means:	11.5	57.25	76.5	83.5	87.0	98.0	103.0	129.25

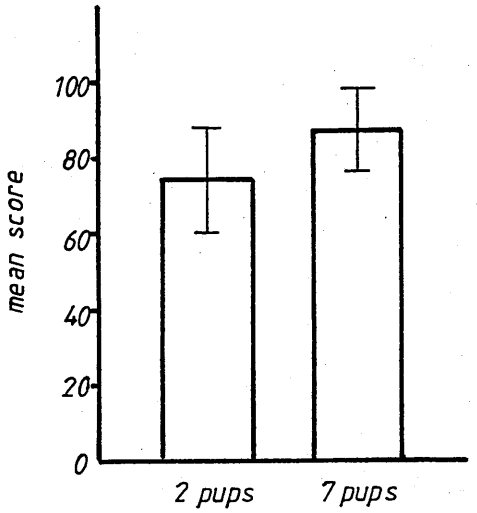


Fig. 4.12a
Litter size

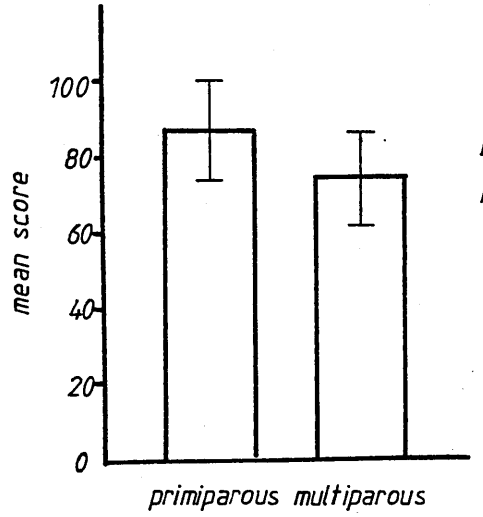


Fig. 4.12b
Maternal experience

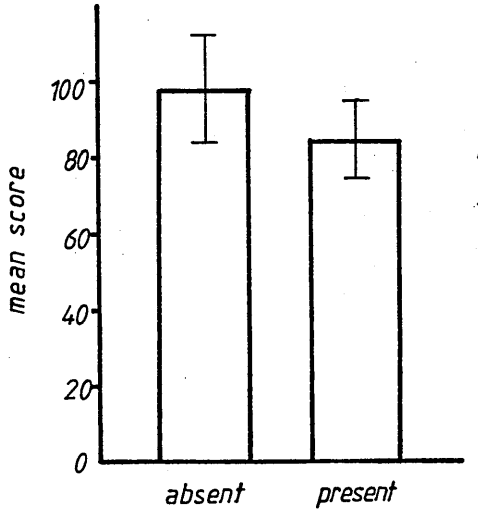


Fig. 4.12c
Paternal presence

Mean scores for open field peripheral compartment ambulation.
Standard errors are indicated.

13. Open Field, Inner Compartment Ambulation

The ANOVA and Scheffé test results are shown in Table 4.13, and the histograms for the means of each main factor averaged over the other main factors are shown in Figs. 4.13a, 4.13b and 4.13c. A significant difference was found with the three-way interaction. The Scheffé test identified five significant differences between pairs of combination means. The 7-Pr-F combination obtained a significantly larger mean value than the 2-Pr-F, the 2-Pr-M, the 2-Mu-M, the 7-Mu-F and the 7-Mu-M combinations. Again there was wide variation within the scores obtained for this test.

Table 4.13

Analysis of Variance of Open Field 'Inner Compartment' Ambulation Scores with Scheffé Test Results

(Based on untransformed data)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	1128.12	0.578	NS
Maternal Experience (M)	1	528.12	0.270	NS
Paternal Presence (P)	1	1378.12	0.706	NS
2-WAY INTERACTIONS				
L x M	1	3120.50	1.598	NS
L x P	1	162.00	0.083	NS
M x P	1	200.00	0.102	NS
3-WAY INTERACTION				
L x M x P	1	1953.12	10.562	<0.01
RESIDUAL	24	184.92		

L x M x P

Combinations:	2-Pr-F	2-Pr-M	2-Mu-M	7-Mu-F	7-Mu-M	7-Pr-M	2-Mu-F	7-Pr-F
Means:	0.5	2.5	3.5	3.75	6.75	14.0	22.75	52.25

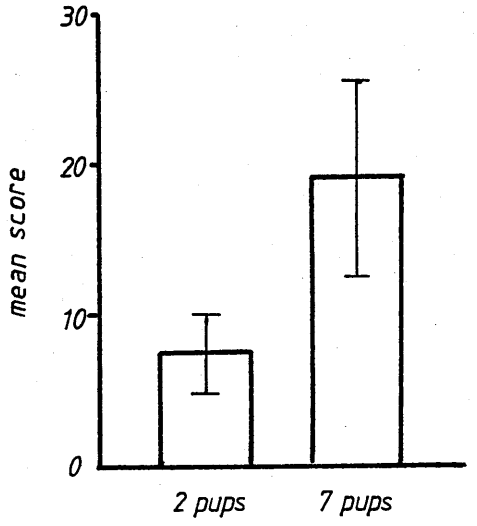


Fig. 4.13a
Litter size

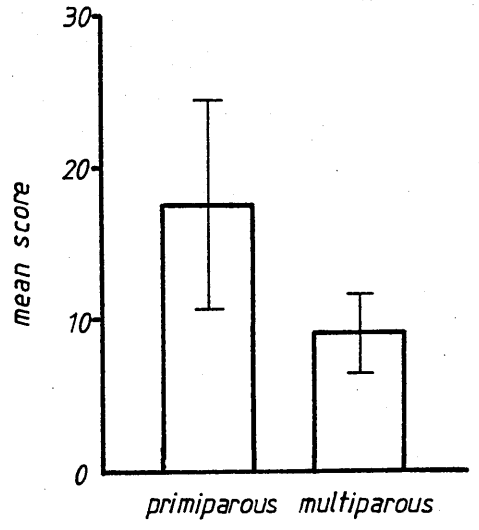


Fig. 4.13b
Maternal experience

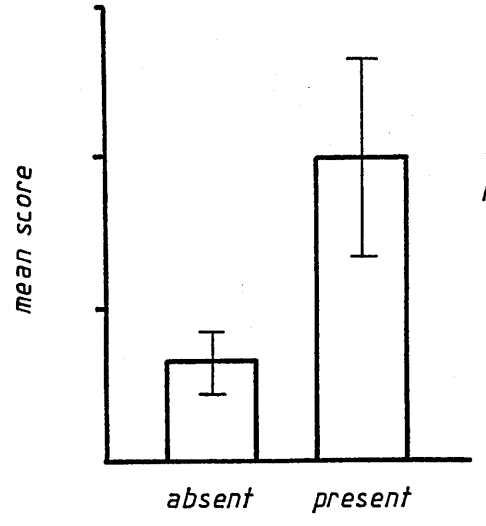


Fig. 4.13c
Paternal presence

Mean scores for open field inner compartment ambulation.
Standard errors are indicated.

14. Open Field, Defaecation

The ANOVA and Scheffé test results are shown in Table 4.14, and the histograms for the means of each main factor averaged over the other main factors are shown in Figs. 4.14a, 4.14b and 4.14c. One main effect is of interest. A significant difference was found between the two levels of the paternal presence variable. When the father was present with the subjects during their pre-weaning development, a significantly higher mean value was obtained than when the father was absent.

Table 4.14

Analysis of Variance of Open Field 'Defaecation' Scores with Scheffé Test Results

(Based on untransformed data)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	4.50	0.521	NS
Maternal Experience (M)	1	8.00	0.927	NS
Paternal Presence (P)	1	50.00	5.794	<0.05
2-WAY INTERACTIONS				
L x M	1	2.00	0.232	NS
L x P	1	8.00	0.927	NS
M x P	1	0.50	0.058	NS
3-WAY INTERACTION				
L x M x P	1	0.00	0.000	NS
RESIDUAL	24	8.63		

P

Paternal Presence:	M	F
Means:	2.75	5.25

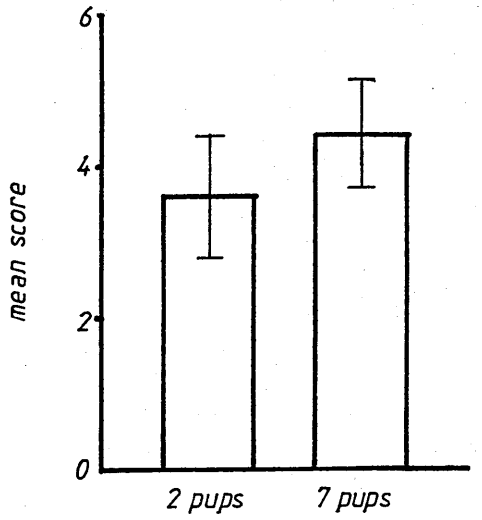


Fig. 4.14a
Litter size

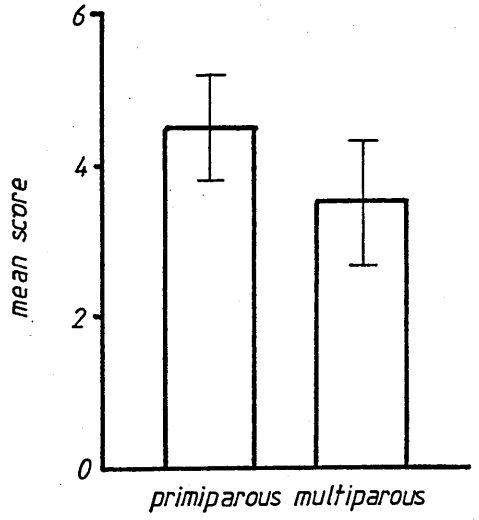


Fig. 4.14b
Maternal experience

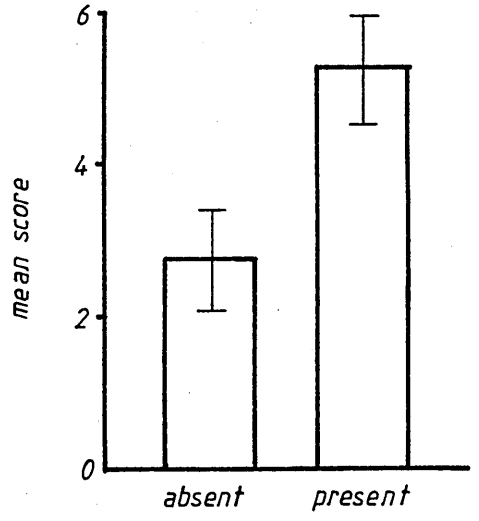


Fig. 4.14c
Paternal presence

Mean scores for open field defaecation. Standard errors are indicated.

15. Body Weight

The ANOVA and Scheffé test results are shown in Table 4.15 and the histograms for the means of each main factor averaged over the other main factors are shown in Figs. 4.15a, 4.15b and 4.15c. Significant differences were found with two main effects. These were litter size and paternal presence.

The effect of litter size was such that pups from smaller size litters were on average heavier than pups from large size litters.

With the paternal presence factor; when the father was present with the developing pups, a significantly larger mean value was obtained than when the father was absent.

Table 4.15

Analysis of Variance of Body Weight Data
with Scheffé Test Results

(Based on untransformed data)

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECTS				
Litter Size (L)	1	59.842	30.661	< 0.001
Maternal Experience (M)	1	4.147	2.125	NS
Paternal Presence (P)	1	40.006	20.498	< 0.001
2-WAY INTERACTIONS				
L x M	1	8.303	4.254	NS
L x P	1	7.334	3.758	NS
M x P	1	0.252	0.129	NS
3-WAY INTERACTION				
L x M x P	1	0.525	0.269	NS
RESIDUAL	24	1.952		

L

Litter Size:	7	2	
Means:	10.24	12.97	*

P

Paternal Presence:	M	F	
Means:	10.49	12.72	*

* measurements in grams

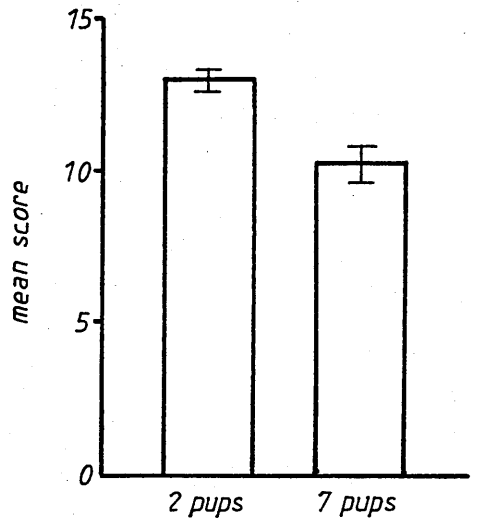


Fig. 4.15a
Litter size

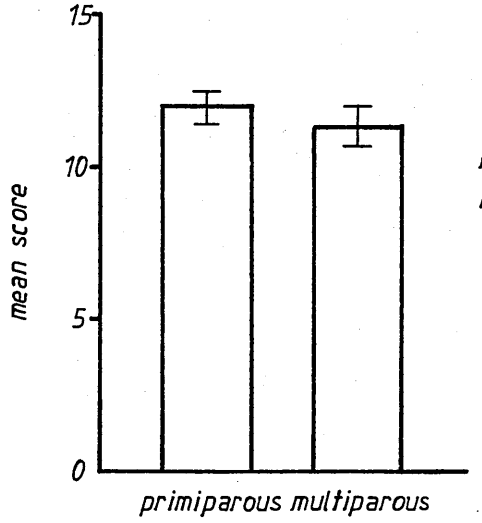


Fig. 4.15b
Maternal experience

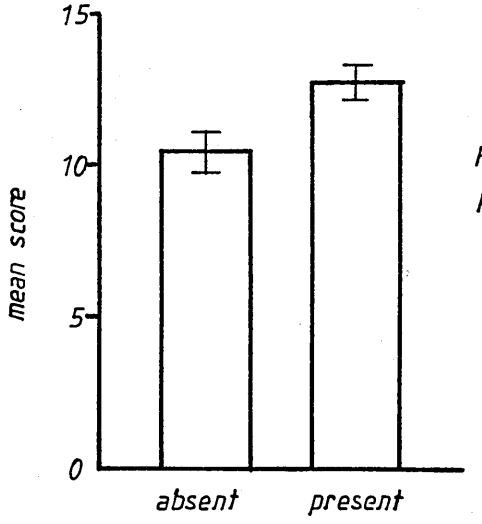


Fig. 4.15c
Paternal presence

Mean body weights [in grams]. Standard errors are indicated.

16. Conclusions and Suggestions for Future Lines of Research

1. The period of time bounded by day 10 and day 25 of the laboratory mouse's pre-weaning period is characterized by a series of rapid developmental changes.

2. For the majority of the recording period, the proportion of time spent by pups in the following activities showed a decrease with pup age:

   (a) time spent in the nest

   (b) proximity to littermates

   (c) locomotor behaviour

3. For the majority of the recording period, the proportion of time spent in the following activities showed an increase with pup age:

   (a) exploratory activities
   (sniffing, head-lifting and rearing)

   (b) grooming

   (c) eating solid food

4. The performance of some of the activities depends upon the development of a quadrupedal stance and locomotion, and certain specific motor skills. The incidence of display of these activities, therefore, could constitute an index for the measurement of physical development.

5. The fact that proximity to both parents was maintained during the pre-weaning period is of interest. It remains to be established what the roles of the parents and offspring are in maintaining this association. Further work, described later in this present study, seeks to explain the role which the father plays in influencing pup development.

6. Some behavioural changes had not stabilized by the age of 25 days when systematic observations of the pups ended. It would be of interest to investigate the future development of these activities.

7. Twelve behavioural activities were recorded on a daily basis during the pre-weaning period; and on day 30, open field tests were conducted, followed by measurements of the pups' body weights. These measures proved to be sensitive to the effect of litter-size, maternal experience and paternal presence on pup development. Only in the case of the 'proximity to mother' and 'locomotor behaviour' activities was there no significant effect caused by any of the three factors. Only one activity; that of 'drinking water'; had to be excluded from the analysis on the grounds that insufficient data were collected.

The significant effects of the three factors and their interactions are discussed in detail in the following chapter.

Chapter Five

THE MAIN EXPERIMENT : DISCUSSION AND CONCLUSIONS

Chapter 5

THE MAIN EXPERIMENT : DISCUSSION AND CONCLUSIONS

In Chapter 4, the results of the main experiment were presented, behaviour by behaviour. In this chapter, the results are discussed, effect by effect, using the following order :

1. Main effects
2. Two-way interactions
3. Three-way interactions

Figs. 5.1 to 5.3 present the mean scores of behaviours for which there were significant main effects. Figs. 5.4 and 5.5 present the mean scores of behaviours for which there were significant two-way interactions. Fig. 5.6 presents the mean scores of behaviours for which there were significant three-way interactions. Histograms of daily measurements are based on transformed data and histograms of day 30 measurements are based on untransformed data. Table 5.1 presents a summary of all the significant differences in the main experiment results. The same abbreviations are used, as those in Chapter 4.

Before looking in detail at the results, it is of interest to note some general patterns. Of the three independent variables; litter size, maternal experience and paternal presence; paternal presence was the variable with the greatest number of significant main effects. Paternal presence significantly affected the scores obtained for head-lifting, rearing, eating solid food, defaecation in open field apparatus, and body weight measurements on day 30. The litter size factor however, had nearly as many significant main effects as the paternal presence factor. Four measurements were affected by litter

Fig. 5.1a Proximity to father

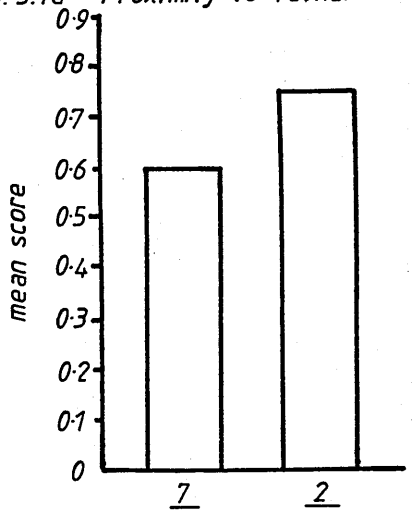


Fig. 5.1b Head-lifting

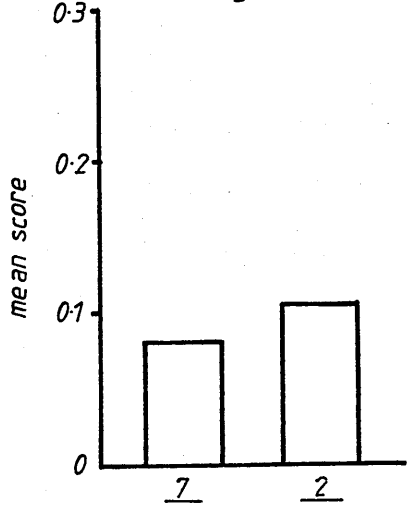


Fig.5.1c Suckling

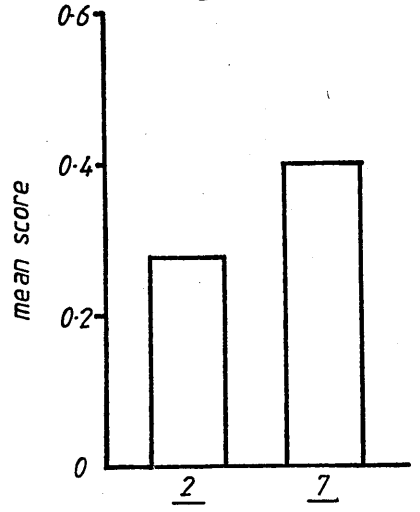
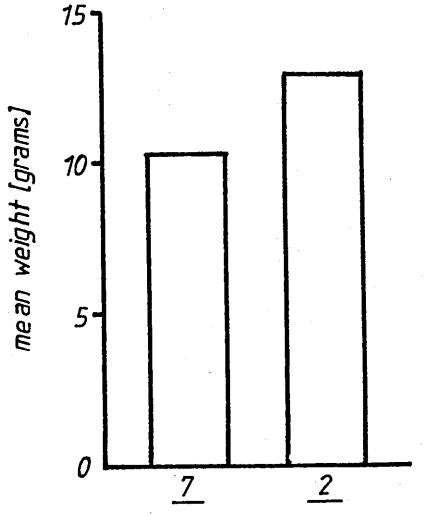


Fig. 5.1d Body weight



Significant litter size main effects [ANOVA]. Overall means are shown. Any two litter sizes not underlined by the same line are significantly different [Scheffé test].

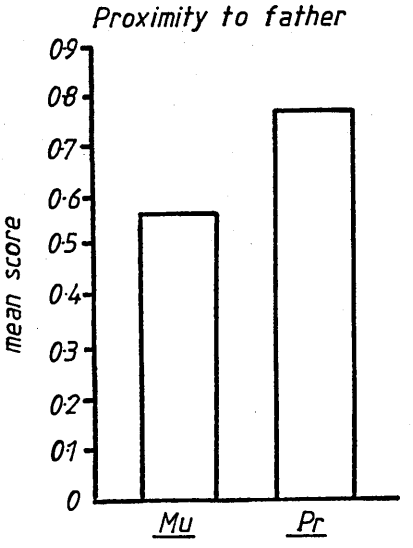


Fig. 5.2 Significant maternal experience main effect [ANOVA]. Overall means are shown. Maternal experience levels not underlined by the same line are significantly different [Scheffé test].

Fig. 5.3a Head-lifting

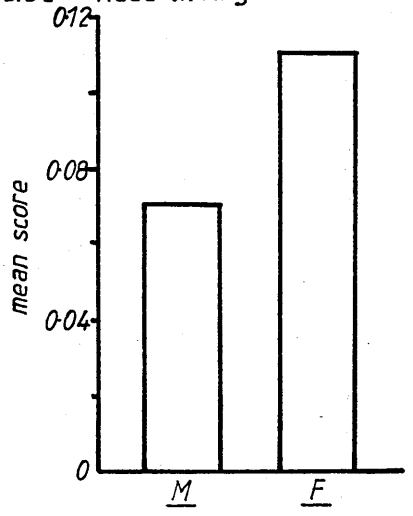


Fig. 5.3b Rearing

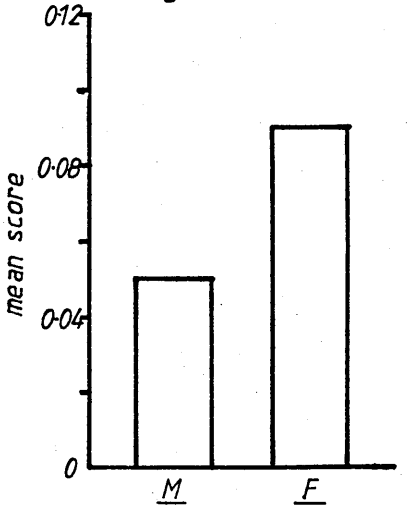


Fig. 5.3c Eating solid food

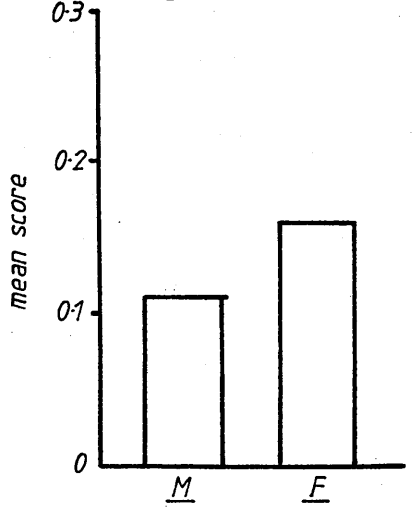


Fig. 5.3d Open field defaecation

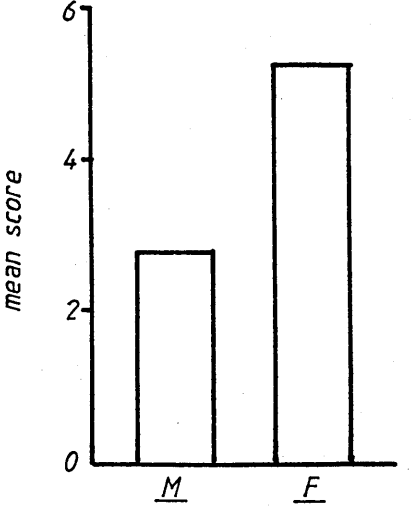
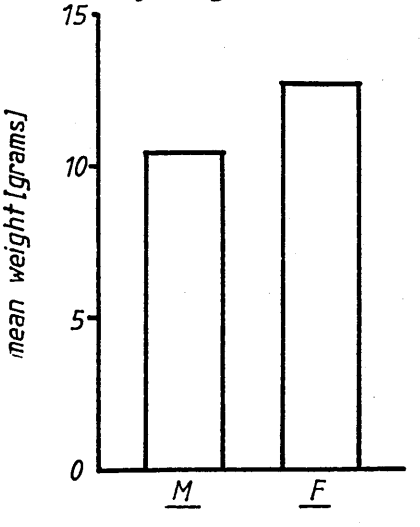


Fig. 5.3e Body weight



Significant <u>paternal presence</u> main effects [ANOVA]. Overall means are shown. Paternal presence levels not underlined by the same line are significantly different [Scheffé test].

Fig. 5.4a

In nest

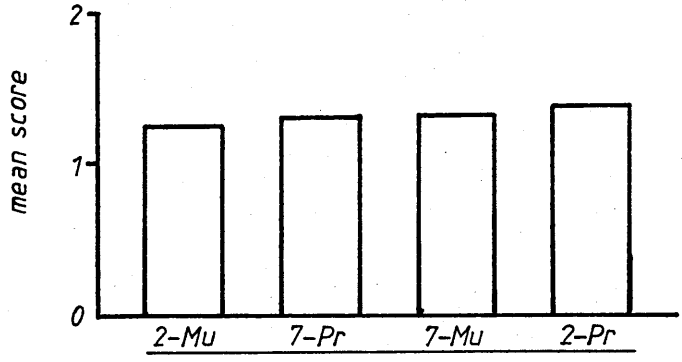


Fig. 5.4b

Proximity to littermate

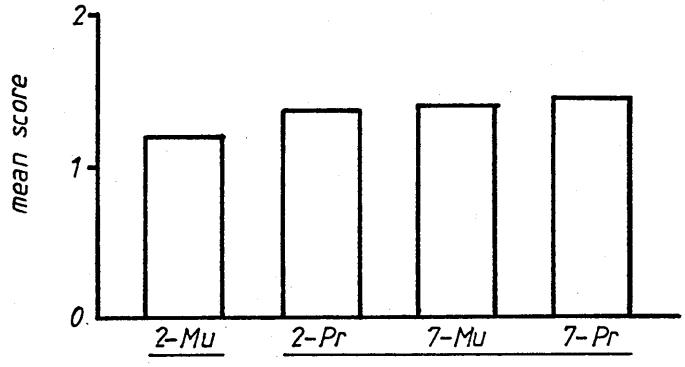
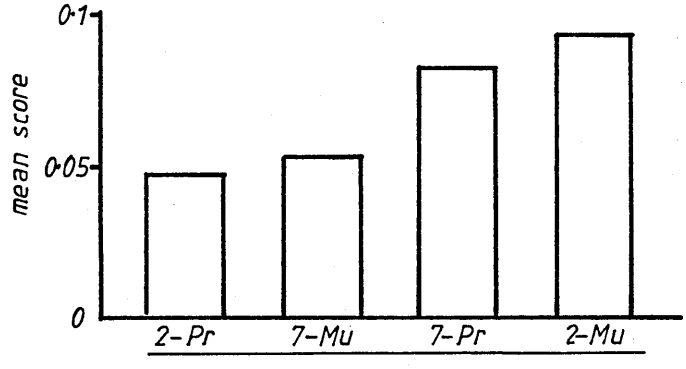


Fig. 5.4c

Rearing



Significant litter size x maternal experience interactions [ANOVA]. Overall means are shown. Combinations not underlined by the same line are significantly different. Combinations underlined by the same line are not significantly different [Scheffé test].

In nest

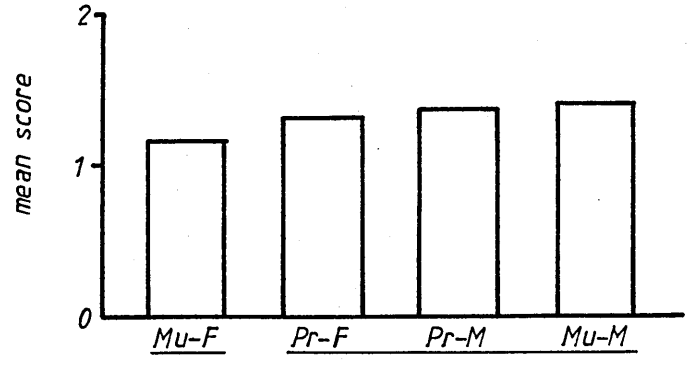


Fig. 5.5 Significant maternal experience x paternal presence interaction [ANOVA]. Overall means are shown. Combinations not underlined by the same line are significantly different. Combinations underlined by the same line are not significantly different [Scheffé test].

Fig. 5.6a Sniffing

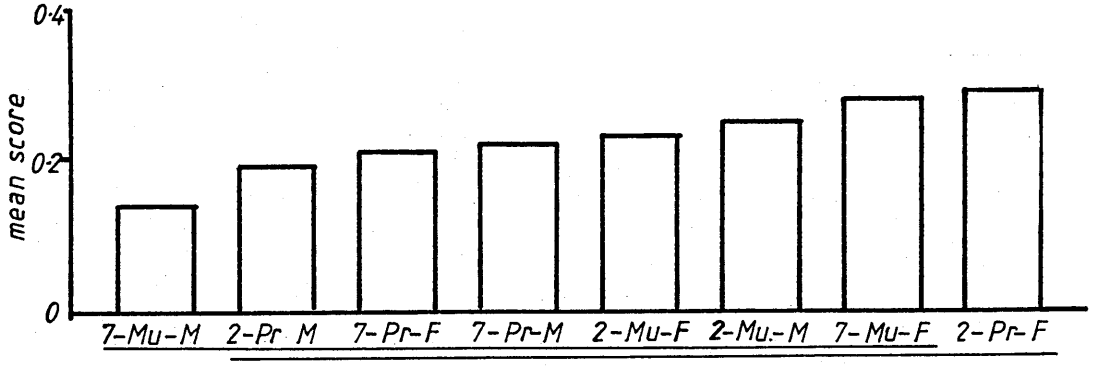


Fig.5.6b Grooming

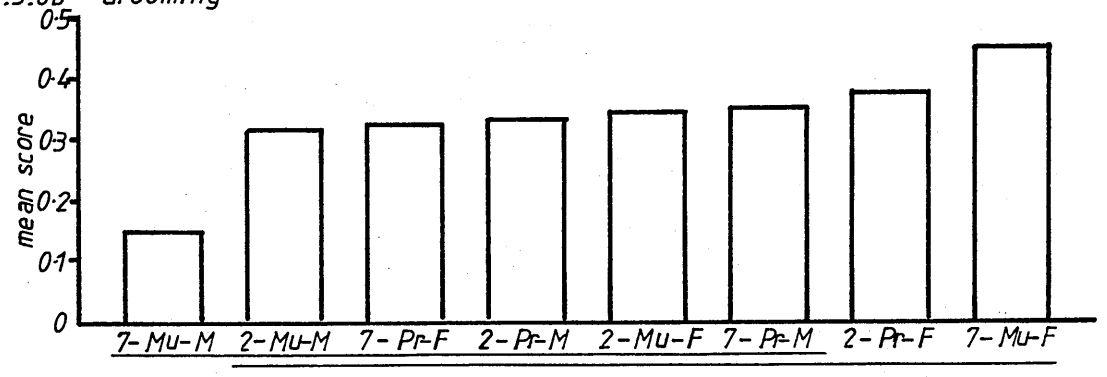


Fig.5.6c Open field peripheral compartment ambulation

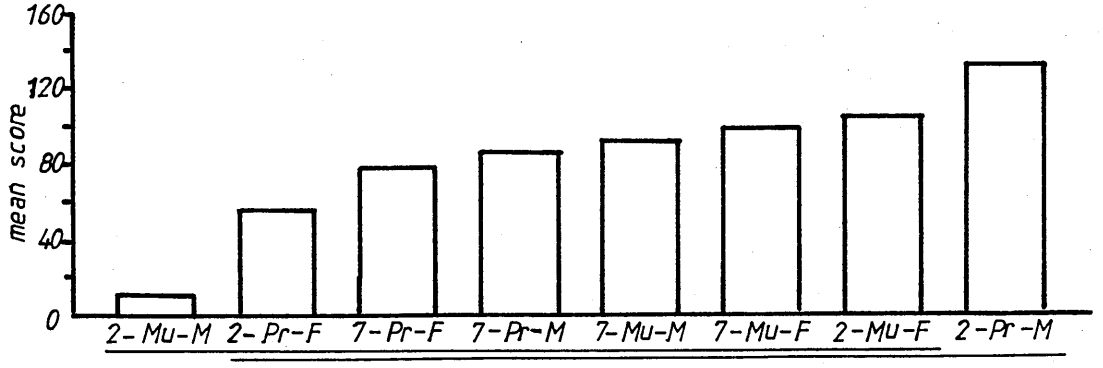
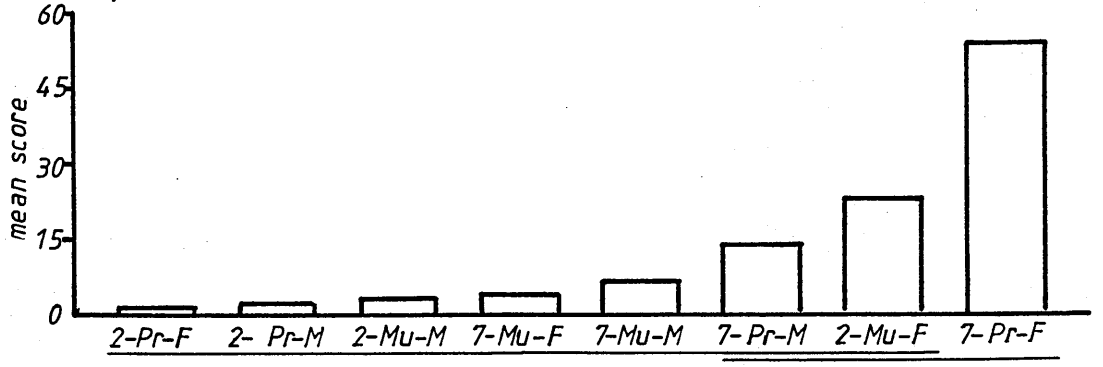


Fig.5.6d Open field inner compartment ambulation



Significant litter size x maternal experience x paternal presence interactions [ANOVA]. Overall means are shown. Combinations not underlined by the same line are significantly different. Combinations underlined by the same line are not significantly different [Scheffé test].

Table 5.1

Summary of Significant Differences in the Results From the Main Experiment

MEASURE	SIGNIFICANT DIFFERENCE	ANOVA P VALUE
Litter Size (L)		
Proximity to Father	7 < 2	<0.05
Head-lifting	7 < 2	<0.05
Suckling	2 < 7	<0.05
Body Weight	7 < 2	<0.001
Maternal Experience (M)		
Proximity to Father	Mu < Pr	=0.01
Paternal Presence (P)		
Head-lifting	M < F	<0.01
Rearing	M < F	<0.05
Eating Solid Food	M < F	<0.05
Open Field Defaecation	M < F	<0.05
Body Weight	M < F	<0.001
L x M		
In Nest	2-Mu < 2-Pr	<0.05
Proximity to Littermate	2-Mu < OTHERS	<0.05
Rearing	2-Pr < 2-Mu	<0.05
M x P		
In Nest	Mu-F < OTHERS	<0.05
L x M x P		
Sniffing	7-Mu-M < 2-Pr-F	<0.001
Grooming	7-Mu-M < {2-Pr-F, 7-Mu-F}	<0.01
Periph.Comp.Ambulation	2-Mu-M < 2-Pr-M	<0.05
Inner Comp.Ambulation	{2-Pr-F, 2-Pr-M, 2-Mu-M, 7-Mu-F, 7-Mu-M} < 7-Pr-F	<0.01

size; and these were proximity to father, head-lifting, suckling and body weight measurements on day 30. The maternal experience factor had only one main effect. The activity affected by maternal experience was that of proximity to the father.

Whereas maternal experience appears less influential than the other two factors when considering the main effects, an examination of the two-way interactions reveals that it was involved in more interactions than either of the other two factors. The combination involving litter size and maternal experience had the largest number of significant two-way interactions, affecting the 'in nest', 'proximity to littermate', and the 'rearing' scores. Only one significant interaction was found with the combination involving maternal experience and paternal presence. This was for time spent in the nest. No significant interactions were found with the combination involving litter size and paternal presence.

Four out of the total of 15 analyses involved significant three-way interactions. These four were 'sniffing', 'grooming', 'open field peripheral compartment ambulation' and 'open field inner compartment ambulation'. The existence of these significant two-way and three-way interactions justifies the experimental approach of investigating the possibility of interactions between the three factors, in addition to the main effects. There is the indication that mouse pup development is characterized by some complicated relationships between the three social factors. These three variables are not operating independently and each must be considered in the light of the other two. By what potential routes, however, might the three main factors and their interactions exert their effect ? In order to lay a basis for the interpretation of the results, possible causal

mechanisms are postulated in this next section.

Possible Effects of the Three Factors

Paternal Presence. The close presence of the father during the pre-weaning period of the pups could alter the pups' environment considerably. It seems that with the father present, the offspring would be likely to receive a more comprehensive and efficient caretaking service (Jakubowski & Terkel 1982; Leblond 1940; Noirot 1964b, 1969b; Priestnall & Young 1978). By being present with the offspring in the nest (Elwood 1983), the father would provide tactile stimulation and thermal insulation. With the father present, it is possible that the pups would copy his adult behaviour (Mugford & Nowell 1972) so that their developmental behaviour was changed as a result. It is also likely that offspring would be exposed to the odours of the father and these could affect developmental rates (Fullerton & Cowley 1971).

The father could also influence the pups through the mediation of the mother. His presence could direct the mother's attention away from the offspring and towards himself, thus depriving the pups of maternal care. He might alternatively relieve the caretaking load of the mother and, in turn, reduce the stress of the mother (Elwood & Broom 1978) with a consequent positive effect on the pups.

Litter Size. The small and large litters would represent completely different environments for the constituent pups. When parents are out of the nest, individual pups are likely to lose less body heat in large litters, compared to small litters. Such changes are believed

to affect development in mice (Barnett & Borland 1967). The reception of tactile stimulation from littermates would tend to be much greater in the large litters than in the small ones, and this has been shown to affect the development of BALB/c mice (LaBarba et al. 1974). The large litters would also be likely to produce more tactile stimulation-induced ultrasounds (Okon 1970b) and fewer cold-induced ultrasounds (Okon 1970a) than the small litters. The former appear to cause adults to withdraw from the pups and the latter appear to attract adults (Noirot 1972b). Adults are also affected by pup odours (Noirot 1969c), and there would presumably be a greater concentration of odours with the large litter compared to the small litter. In addition to affecting parental behaviour, the auditory and olfactory output of the litter could affect the constituent pups directly. It would appear that a large litter could promote litter aversion by the mother and a deterioration in maternal care (Seitz 1958); perhaps because of the greater caretaking demands placed on the mother of a large litter, compared to the mother of a small one. On the other hand, if a stimulus threshold is required for caretaking behaviour to be displayed, the large litter is likely to be more capable of providing it than the small litter (Leigh & Hofer 1973). The amount of attention from the parent per pup would tend to be greater in the small litters, compared to the large ones. The quality and quantity of milk obtained per pup in a small litter could conceivably be greater than that obtained in a large litter (Kumaresan et al. 1967).

Maternal Experience. If the multiparous mother's behaviour has been accentuated by her previous breeding experience (Leblond 1940), then consequent changes in the offspring's development might be noticed. It is possible that the multiparous mother would be more resilient to large caretaking demands placed on her and be more ready to display

maternal behaviour than primiparous animals (Seitz 1958). Alternatively, greater experience with offspring could be equated with diminished performance of some maternal behaviour, as Noirot (1964b) found. Changes in maternal experience could also affect the mother's relationship with the father, which might have its repercussions in terms of the father's relationship with the offspring.

1. Main Effects

Litter Size

In comparison with pups in large litters, small litter pups obtained significantly larger 'proximity to father', 'head-lifting' and 'body weight' scores, and significantly smaller 'suckling' scores (Fig. 5.1).

The 'proximity to father' result (Fig. 5.1a) indicates that the father was either more attracted to smaller litters or that he showed an aversion to larger ones. It has been reported that mothers with small litters spent more time in the nest than those with larger litters, in the laboratory rat (Grota 1973; Grota & Ader 1969; Leigh & Hofer 1973) and in the laboratory mouse (Priestnall 1972). I did not find a significant litter-size effect on 'proximity to mother' scores but similar mechanisms could have been operating to cause this effect in the father. Seitz (1958) found that in primiparous rats there was a stepwise inverse correlation between litter size and the display of maternal behaviour. He suggested that this behavioural trend was either caused by excessive fatigue in the mothers of large litters or served to protect the mothers of large litters from excessive fatigue.

This same theory could be used to explain the father's behaviour, especially if the father was playing an active caretaking role. Another explanation is that cold-induced ultrasounds attracted the father to the pups (Noirot 1972b). Elwood & Broom (1978) suggested that the higher incidence of pup ultrasonic calling in small litters of gerbils (Meriones unguiculatus), compared to large litters, could attract the mothers to the pups. This is, however, unlikely to explain the 'proximity to father' result, since this litter size effect was apparent after day 15 (Fig. 4.4a) when the pup ultrasonic calls are likely to be decreasing due to age (Okon 1970a). The larger 'proximity to father' scores of the small litters suggests that they received more parental tactile stimulation and more parental insulation against heat loss than large litters. It is of interest to note that Elwood & Broom (1978) also found a litter size effect on the time spent by gerbil pups with the father. They also found that fathers with small litters (one pup) spent significantly more time in contact with the offspring than fathers with large litters (three and five pups).

Two explanations are offered for the 'head-lifting' result, where the small litters obtained a significantly greater mean score than the large litters (Fig. 5.1b). It is to be expected that pups in a large litter would be engaged in more inter-pup social interactions than those in a small litter. Large-litter pups would spend a large proportion of time contacting each other, crawling over each other or avoiding each other. Small-litter pups would not be involved in as many inter-pup interactions and consequently would have more opportunity for alternative activities such as head-lifting. The other explanation concerns the small litters' greater 'proximity to father' scores

compared to the large litters. The closer association of the father and offspring might promote larger investigative scores in the offspring. The question must be asked, however, why did this not cause an interaction between litter size and paternal presence (L x P) to emerge ? Since the father was present in only half of the cases, the 2-F combination would be expected to provide a mean score which was significantly larger than the means of the other three L x P combinations (2-M, 7-M and 7-F). An L x P interaction was not found however.

With the 'suckling' result, the small litter obtained a lower mean score than the large litter (Fig. 5.1c). It should be remembered that suckling was scored for both approaching and engaging the nipple as well as for actually ingesting milk. (This activity is defined and described in Chapter 2.) With a litter size of two pups, each pup would tend to locate a nipple and begin feeding very quickly. The larger litter size of seven pups would be likely to cause an extension of the period of time any single pup would need to locate a nipple. With a larger litter the pups would very likely get in each others' way, mutually preventing free access to the nipples. This extended duration of nipple-seeking would then be reflected in the suckling scores.

Fig. 4.10a shows that the difference in suckling scores between the two litter sizes was apparent throughout the recording period. For this reason, low suckling scores cannot be equated with high 'eating solid food' and 'drinking water' scores; neither of which were displayed until the end of the recording period. Further, both the 'eating solid food' and the 'drinking water' results showed an absence of significant differences between the two litter sizes.

The different suckling scores may reflect the rate at which the pups can obtain milk from the mother. Kumaresan et al. (1967) indicated that female rats produced less milk when nursing small litters, but that there was more milk available per pup, compared to large litters. If this greater quantity of milk could be transferred more quickly, it would result in faster pup satiation and shorten the suckling duration. This suggestion, however, needs to be viewed in the light of the findings of Eisen et al. (1977). They observed that laboratory mouse mothers nursing smaller litters, had smaller mammary glands. This would tend to delay pup satiation and prolong the suckling duration. Newosielski-Slepowron & Park (1977) found that up to an optimum litter size of approximately seven pups, the lactational capacity of rat mothers increased relative to the litter size. This would suggest that slower milk ejection rates in mothers of larger litters were not the cause of prolonged suckling time in their pups.

As indicated in Chapter 2, there is the danger in very small litters that there would not be a sufficient suckling stimulus to maintain lactation. The rat mother appears to eject milk in response to a group stimulus from the litter (Drewett et al. 1974) and in small litters the stimulus threshold may not be reached. It could be argued, therefore, that a lower suckling score in the small-litter pups was due, not to satiation, but to depleted milk supply; a reason given by Drewett et al. (1974) for leaving the nipple. This is most unlikely, however, since the body weight figures, discussed below, indicate that the pups from the small litters were not nutritionally disadvantaged. If the growth of the pups was determined by the quantity of the mother's milk they received (MacDowell et al. 1930), the small-litter pups were obtaining more milk than the large-litter pups.

In contrast to this finding that pups in small litters spent less time suckling than pups in large litters, Elwood & Broom (1978) found that in gerbil pups it was the other way round. Priestnall (1970, 1972) also found that mouse pups were suckling for a larger proportion of time when in small litters. It is possible, although by no means certain, that this difference between my own findings and those of the above workers was due to the adoption of differing methods for scoring suckling. For the reasons provided earlier, I decided to include nipple-seeking with suckling. If variations in the time taken for different litter-size pups to reach the nipple were responsible for my own results, comparisons between these and other workers' results are limited, but support is provided for the 'competition for nipple sites' hypothesis.

Comparisons of body weights on day 30 revealed that pups from small litters had, overall, higher body weights than those from large litters (Fig. 5.1d). This result may indicate that the small litters neither suffered from nutritional deficiencies nor experienced inhibited physical development when compared to the large litters. In fact the converse is suggested, although other reliable indices of physical development would be required to confirm this.

An explanation for the body weight differences may be found in the quality of the milk received by the pups in the two different size litters. Hill (1972) suggested the possibility of an association between the quality of the mothers milk and the growth rate of white-footed mouse pups (Peromyscus leucopus). It is possible that mothers with larger litters, and faced with the requirement to provide a larger volume of milk, may as a consequence provide milk which is less concentrated and which is of poorer quality than mothers with smaller

litters. Such a difference in milk quality would be likely to be reflected in the body weights of developing offspring. Such a conclusion could not be drawn, however, in the absence of physiological support.

Since body weight is a measure of physical development, it is also possible that some characteristic feature of the small litter situation actually accelerated development. If this was the case, a likely candidate would be the amount of tactile stimulation received, since it would appear that the pups in small litters were receiving more attention from the father. Padmanabhan & Singh (1980) suggested that inter-animal relationships were responsible for growth rate differences between animals from different sized rat litters. Winick & Noble (1967) commented on a mechanism that would be likely to affect growth, and claimed that cell division rates were affected and that these determined the final number of cells per organ in developing rats. As already discussed, the thermal characteristics of a litter would be determined, to some extent, by the litter size, and this may also have affected development. In Russell's (1971) review of infantile stimulation, both tactile and thermal effects were considered to be likely instruments for causing behavioural changes in rodent litters; possibly through the mediation of changes in physiological mechanisms. It is possible that a combination of thermal and tactile stimulation factors were responsible for changes in developmental characteristics. Denenberg (1964) put forward a theory of 'total stimulus input' where the sum, or interaction, of the stimuli from various sources was seen to determine the resultant effects. Staying with thermal explanations, Dudley (1974a) concluded that the father's presence was responsible for maintenance of body temperature and, in turn, weight gain in California mouse pups (Peromyscus californicus). This also represents

a possible explanation for my own results. The finding, that mouse pups from small litters attain higher body masses than those from large litters, is consistent with most other reports on the litter-size effect on rodent development. These were reviewed in Chapter 1.

It is of interest that, in contrast to the many reported litter-size effects on open field behaviour reviewed in Chapter 1, no such litter-size effects were found in this experiment. With exceptions, (for example, Elwood & Broom 1978), earlier work has paid far more attention to the effect of litter size on maternal behaviour than on paternal behaviour. My own results shift the emphasis towards the father-pup relationship. Such a relationship could prove to be an important determinant of mouse pup behavioural development. It is possible that increased pup attendance by the father in small litters, compared to large litters, may have contributed to the mediation of other reported effects in this experiment.

Maternal Experience

Only one main effect, due to maternal experience, was found; that is, offspring reared by a multiparous mother obtained a significantly smaller 'proximity to father' mean score than offspring reared by a primiparous mother (Fig. 5.2). Fig. 4.4b shows that its influence was particularly marked towards the beginning of the recording period. The indication is that, since the pups spent most of their time in the nest during this period, the father had been responsible for the effect by spending a larger proportion of time in nest attendance when accompanying a primiparous mother as opposed to a multiparous mother. If this is the case, one could question whether the father was playing a compensatory role for inadequacies in the primiparous mother's

caretaking behaviour. The caretaking roles of the mother and the father are examined in Chapter 6.

In contrast to the effect of maternal experience on 'proximity to father' scores, no corresponding effect was found with the 'proximity to mother' scores. The effect on 'proximity to father' can be interpreted in a number of ways. One possibility is that primiparous and multiparous mothers respond differently to the father which in turn influences the relationship between the father and the offspring. For example, Dice (1929) reported that in some rodent species, the mother frequently prevented the father from having access to the nest during the early pre-weaning period. Horner (1947) however, did not find that the father had this restriction imposed on him in Peromyscus spp.; nor have I ever observed this in the laboratory mouse.

It is possible that differences in the mother's caretaking behaviour, due to maternal experience, had changed the stimulus characteristics of the pups; for example, concerning the emission of ultrasounds; which, in turn, had affected the relationship between the father and the pups. It has been suggested (Harper 1970; Rosenblatt & Lehrman 1963; Schaffer 1977; Spencer-Booth 1970) that the offsprings' effect on the parents is as important as the parents' effect on the offspring.

Another explanation for this 'proximity to father' result concerns the experience of the father. It will be necessary to refer to the father's reproductive and caretaking experience since this will vary in correspondence with the mother's. The composition of each adult pair at the time of initial mating was preserved during subsequent breeding episodes (Chapter 3). It is possible that the fathers which

were with multiparous mothers, spent less time with the pups because they were more experienced than the fathers accompanying primiparous mothers. In the past much emphasis has been placed on the maternal experience factor with little consideration for an equivalent paternal experience effect. Some evidence from previous experimental work was provided in Chapter 1 to support the idea that differences in maternal behaviour exist between primiparous and multiparous rodents. It is therefore conceivable that the significant difference between the two 'proximity to father' means could have been caused by differences in the mother's behaviour influencing the father-pup relationship. Alternatively, it could have been caused by differences in the father's behaviour directly affecting the amount of contact time with the offspring.

In the original design of this experiment, maternal experience was emphasized because of the mother's well-known and highly necessary role of providing caretaking behaviour (Denenberg 1972; Harper 1970; Thoman & Arnold 1978). The general observations and results strongly indicate however, that the experience of the father must also be considered. It must be remembered that it was the 'proximity to father' results which were affected by this treatment and not the 'proximity to mother' results. One might also expect the 'suckling' results to be significantly affected by maternal experience, but this was not the case. Further work is now called for, in order to unravel the maternal experience effect from the paternal experience effect. A 2 x 2 design, involving two levels of maternal experience for one independent variable and two levels of paternal experience for the other, could profitably be used.

Having considered the effect of maternal experience on the development of offspring, it is evident that primiparous mothers were sufficiently maternal to enable pup development to proceed without fatalities or noticeable behavioural deficits in the offspring; a general observation also referred to by Richards (1967). Beach & Jaynes (1956a) and Rosenblatt & Lehrman (1963) commented that this was the case in rats; and Lashley (1938) commented that it was true of mice.

Paternal Presence

Five measures were significantly greater in the presence of the father than in his absence. These were 'head-lifting', 'rearing', 'eating solid food', 'defaecation in open field apparatus' and body weight measurements.

Significantly larger head-lifting scores were obtained overall, when the father was present, compared to when he was absent (Fig. 5.3a). It could be argued that the father's presence had caused the mother's attention to be directed towards himself and away from the litter, and that an increase in head-lifting was the pup response to the absence of the mother from the nest. 'Proximity to mother' scores were not significantly affected by paternal presence, however, rendering this 'maternal neglect' hypothesis unlikely. I gained the general subjective impression from casual observations, that when the father was present, pups were likely to benefit from increased, rather than decreased, caretaking. The father was observed spending a large proportion of time in the nest with the pups, and the mother's caretaking behaviour was not seen to suffer when the father was present. It is more likely that head-lifting is an immediate response to the presence of the father, possibly caused by the father's olfactory output. (It should

be noticed that this possible connection between the father's presence and offspring head-lifting, is consistent with the litter-size result, where close proximity to the father was associated with an increased display of head-lifting.) Obvious sources of adult male odour would be the urine and the preputial glands (Brown & Williams 1972). Bronson & Caroom (1971) found that male urine, the odour of male preputial gland homogenate and lipid extracts from male preputial glands, all constituted an effective attractant for sexually inexperienced female mice. Male mice are also sensitive to adult male odours as is evidenced by odour-induced aggression experiments (Archer 1968; Mugford & Nowell 1971). It remains to be established whether such odours evoke head-lifting behaviour in the recipient animals.

Significantly larger rearing scores were also obtained overall, when the father was present, compared to when he was absent (Fig. 5.3b). It would be tempting to maintain that rearing is another exploratory activity like head-lifting, and that this response could be explained as a response to the father's presence, in the same way as the head-lifting result. Rearing also serves as a preliminary to climbing and feeding from the cage-lid food container, however, and on this basis it is possible that those animals which were displaying most rearing were in fact doing so because their rate of development was accelerated. Dudley (1974a) found that California mouse offspring raised in the presence of the father showed advanced development in comparison with those raised without the father. Dudley considered that this was because the father helped to reduce the rate of heat loss by the pups. Elwood & Broom (1978) also found that gerbil offspring exhibited faster behavioural development when the father was present. They also supported the suggestion that the major

contribution of the father to the offspring was a thermal one. Priestnall & Young (1978), however, did not find that the laboratory mouse offspring developed faster in the presence of the father, compared to offspring raised without the father; even though they acknowledged that pups raised with the father received some protection from heat loss. An alternative developmental rate hypothesis to the thermal one, involves the reception of tactile stimulation. Levine (1962b) discussed a 'direct action' hypothesis where stimulation, perceptible to the pups, would influence the physiological systems of the offspring which were undergoing rapid development. Pups raised with the father would be likely to receive more tactile stimulation than those raised without the father. Although Cross & LaBarba (1978) found no evidence to support the claim that neonatal stimulation leads to accelerated physical development in BALB/c mice, LaBarba et al. (1974) found that it affected their behavioural development. Other findings have supported this claim, however (for example, Denenberg & Karas 1959), and it has been suggested that there may be optimum degrees of stimulation which are required for development to proceed normally (Denenberg 1962b) or for it to develop more rapidly than usual (Levine 1962a). It may be that with only the mother present, the amount of stimulation is less than optimum, but that the optimum level is reached with the presence of both parents.

Pups reared with the father spent, overall, a significantly larger proportion of time eating solid food than pups without the father present (Fig. 5.3c). This may again be a result of faster development in the 'father present' pups since the onset of this activity constitutes a milestone in mouse pup development. 'Suckling' score differences might be expected to provide an inverse correlate of 'eating solid food' scores but no significant paternal presence effect was found

with suckling. In common with the spiny mouse (Acomys cahirinus) (Porter et al. 1980), suckling in my own mice continued until long after solid food was first ingested. This would indicate that the pups raised in the presence of the father were getting the best of both nutritional worlds, and this may help to explain the body weight data discussed below.

Animals reared with the father obtained significantly larger defaecation scores, overall, than those reared without the father (Fig. 5.3d). Defaecation in open field tests is generally taken to indicate a level of emotionality in subjects. High defaecation scores have been associated with low ambulation scores (Denenberg 1963a) and both have been regarded as indicators characterizing a high level of emotionality (Broadhurst & Levine 1963; Denenberg 1963a; LaBarba & White 1971). It has been claimed that frequent defaecation represents a maladaptive stress response which has been mediated by the autonomic nervous system (Denenberg 1963a; Levine 1960). If defaecation scores provide a measure of anxiety or emotionality, those mouse pups raised in the presence of the father displayed high levels of anxiety. As indicated earlier, however, this conclusion could not be supported by observations of the relationship between the father and the pups. Pups appeared to receive more parental care and attention when the father was present, and fathers were not observed antagonizing offspring.

The significant defaecation difference was not associated with any other significant open field test difference. This measure may not, therefore, provide a valid index of anxiety levels. Daly (1973) and Denenberg, Wehmer, Werboff & Zarrow (1969) questioned whether mouse defaecation scores validly measured emotionality and Villescas et al. (1977) claimed that the open field defaecation measure had not been

found to have a relationship with other supposed measures of emotionality. Brain & Nowell (1969) and Bruell (1963) maintained that defaecation in mice may be associated with territorial marking, and Saylor & Salmon (1971) found that BALB/c mice obtained group mean scores for open field defaecation which were inconsistent with that assumed by the general emotionality hypothesis. That is, low defaecation rates were not accompanied by high activity rates.

Experiments have been conducted which have linked increased pre-weaning tactile stimulation with elevated open field defaecation scores in both the rat (Caldwell & Kesner 1966) and the mouse (Henderson 1964). Such results are consistent with my own since the father's presence in my own experiment would have provided the offspring with extra tactile stimulation. The only conclusion which can be confidently drawn from this result, however, is that the paternal presence treatment significantly affected the defaecation response of the offspring to a novel environment.

The body weight result supports the suggestion that the presence of the father accelerated pup development. A significantly greater mean mass was obtained for the groups raised with the father, compared to those raised without the father (Fig. 5.3e). It would seem likely that, since the father-present pups spent more time ingesting solid food than the father-absent pups, they would also experience greater weight gains. There are a number of ways by which the father could have caused this body weight result. Olfactory, tactile, thermal, maternal substitution and maternal mediation hypotheses are the most relevant and will be discussed in turn.

Fullerton & Cowley (1971) found that young female mice reared in the presence of adult males showed accelerated development. The ages at which the eyes opened, the ears opened, the incisors erupted, the pinnae unfolded and the vagina opened were earlier than in control animals which were not exposed to adult males. The father's presence also had the effect of accelerating weight gain in the developing mice. Fullerton & Cowley argued that adult male odours were likely to be responsible, since the males were not in contact with the pups. It is conceivable that in my own experiment, it was the father's odours which were instrumental in accelerating weight gain.

Another possibility is that the father was responsible for promoting weight gain in pups by increasing the amount of stimulation the pups received. As indicated earlier, the suggestion is that a physiological mechanism had mediated changes affecting later behaviour and development (Ader 1970; King & Eleftheriou 1959; Levine 1959b; Russell 1971).

The thermal hypothesis has already been discussed and it has the support of researchers working with the gerbil (Elwood & Broom 1978; Waring & Perper 1980), the white-footed mouse (Hill 1972), the California mouse (Dudley 1974a), the laboratory mouse (Mugford & Nowell 1972), and other mouse species (Chew & Spencer 1967). The father's presence in the nest would inhibit heat loss (Barnett & Borland 1967; Barnett & Manly 1958), and in turn help to ensure that food energy was directed away from temperature maintenance and towards growth. Dudley (1974a) found that when California mice were deprived of their mothers, those pups which were with their father experienced higher body temperatures than when the father was also absent. It seems highly likely that,

with the father present, metabolic rates can fall to a lower level without reduction of body temperature; a mechanism which would reduce the amount of thermal energy lost and which would tend to increase the body weight. It is claimed that mice can thermoregulate properly at the time their eyes open (Chew & Spencer 1967). From this time onwards the father's presence could significantly reduce the proportion of energy in the ingested food which is lost in temperature maintenance.

The father may have also affected the pups by adding to or substituting for the mother's caretaking behaviour. The father, it seems, can perform most of the caretaking tasks of the mother except for that of nursing. Dudley (1974b) suggested that the father played a caretaking role in the rearing of young California mice. Urogenital stimulation, which is necessary for elimination, can be performed by the father in gerbils (Elwood 1975a).

The maternal mediation hypothesis was considered in Chapter 1 and is the subject of two experiments in Chapter 6. The suggestion made is that the father's presence affects the mother's behaviour which, in turn, causes changes in the offspring's development. Dudley (1974a) suggested that the father's presence could have improved the mother's milk supply, either through allowing the mother to spend less time with the litter and more time feeding herself, or through a neuro-endocrine mechanism. This is an important hypothesis to investigate. Smotherman & Bell (1980) suggested that the confounding of direct and maternally mediated influences has prevented a full understanding of the way an offspring's experiences affect its later behaviour.

2. Two-Way Interactions

Litter Size x Maternal Experience

Three, two-way interactions involving litter size and maternal experience were significant. The 2-Mu offspring obtained the smallest mean score for 'in nest' and 'proximity to littermate' activities, and the largest mean score for 'rearing'. All three of these results can be explained by suggesting that the interaction of small litter size and multiparous mother resulted in faster offspring development. Evidence was reviewed in an earlier part of this chapter to suggest that maternal behaviour was affected by parity. It is possible that the more experienced mothers caused their offspring to develop at a faster rate than primiparous ones; but that this only became apparent in small litters where the mother was able to devote more of her time to each pup, rather than in large litters where pups had to compete more for the mother's attention.

For the 'in nest' measure, the 2-Mu mean was significantly smaller than the 2-Pr mean (Fig. 5.4a). Pups which were developing faster might conceivably spend more time out of the nest and, as a consequence of this, spend less time in close proximity to their littermates. The 2-Mu mean for the 'proximity to littermate' measure was significantly smaller than the other three means; which were the 2-Pr, the 7-Pr and the 7-Mu means (Fig. 5.4b). It is, of course, possible that instead of the 'in nest' effect causing the 'proximity to littermate' effect, it was the other way round. Development in laboratory mice involves the establishment of a gradual independence from littermates (Williams & Scott 1953). A proclivity to move away from littermates would result in a decrease in the 'in nest' scores.

The 'rearing' analysis showed that the 2-Mu mean was significantly greater than the 2-Pr mean (Fig. 5.4c). This is to be expected if the 2-Mu offspring were exhibiting accelerated development. As indicated earlier, rearing is an activity including both an investigative element and a preparatory element for other motor skills; namely climbing and obtaining solid food and water. If the mother's behaviour was responsible for the offspring's accelerated development, what aspect of her behaviour caused this ? Cross & LaBarba (1978) found that maternal behaviour in BALB/c mice did not show a strong correlation with offspring developmental rates. Ward (1980) found that the pattern of maternal manipulation of laboratory mouse pups which caused their accelerated development, involved more than just the overall quantity of manipulation. Further conclusions cannot be drawn until the mother's treatment of the offspring has been investigated more closely.

Maternal Experience x Paternal Presence

One significant interaction was found and this involved the 'in nest' activity. The Mu-F pups spent, on average, a smaller amount of time in the nest than each of the other three groups of pups : Pr-M, Pr-F and Mu-M (Fig. 5.5). This result indicates that pups reared by a multiparous mother and with a father present, experienced faster development. Elwood & Broom (1978) found that gerbil pups spent less time in the nest when the father was present. It is possible that in my own experiment, the multiparous mother had responded to the father's presence by behaving towards the pups in a manner which encouraged their departure from the nest. This suggests that it was the more experienced mother which was sensitive to paternal presence. It is also possible that the offspring's behaviour was altered by the presence of the father, and that this changed behaviour evoked a different response

from the multiparous mother which, in turn, caused developmental changes in the offspring. Barnett & Burn (1967), Richards (1966a) and Sherrod et al. (1974) have indicated that handling effects on offspring may be mediated by maternal behaviour. It is important now to consider whether paternal presence effects are mediated in the same way.

3. Three-Way Interactions

There were four significant litter size x maternal experience x paternal presence (L x M x P) interactions. These involved 'sniffing', 'grooming', 'open field peripheral compartment ambulation' and 'open field inner compartment ambulation'. Two combinations of factors are seen to be particularly important. The 2-Pr-F combination resulted in larger 'sniffing' and 'grooming' means and a smaller 'inner compartment ambulation' mean, than the other combinations. The 7-Mu-M combination resulted in smaller 'sniffing', 'grooming' and 'inner compartment ambulation' means than other combinations. The 2-Pr-F pups spent a greater proportion of time sniffing than the 7-Mu-M pups (Fig. 5.6a). Sniffing is the first exploratory mode to be exhibited by the pups. It requires less effort than head-lifting and rearing and, because of its association with olfactory stimuli, can provide pups with information long before their eyes open; around or just after day 12 (Williams & Scott 1953). This should again direct our attention to the olfactory influence of the father on the pups, which has already been discussed. It is of some significance that the highest scoring combination in this analysis is one with the father present. The small litter-size is another aspect of this high scoring combination of factors. It is possible that in a litter of two, adult male odours are more noticeable to the pups. A large litter would be more likely to mask the father's scent with its own. The fact that

the primiparous mother is involved in the high scoring combination is not unexpected and is probably linked to the 'proximity to father' scores where it was seen that Pr pups spent a larger proportion of time in close proximity to the father. If the father's close presence evoked a sniffing response in pups due to his odours, it would be predicted that the Pr pups would be observed sniffing more than Mu ones. The combination of small litter-size, a primiparous mother and the presence of the father caused pups to spend the largest proportion of time sniffing, compared to other combinations. It is likely that the 7-Mu-M combination resulted in the smallest mean value for sniffing because it lacked all three of the components which have been associated with high sniffing scores.

With grooming, it was once again the 2-Pr-F offspring which obtained a significantly larger mean score than the 7-Mu-M offspring (Fig. 5.6b). A similar mechanism to that operating for sniffing may be at work, therefore. This would suppose that the primiparous mother's behaviour again mediated a paternal presence effect and caused pups to groom more when the father was present. Large grooming scores have been considered to represent a particular emotional response (Robinson 1963). Alternatively, increased grooming may represent faster development of muscular control and co-ordination (Fentress 1978).

When the open field 'peripheral compartment ambulation' scores were analyzed, it was found that the 2-Pr-M mean was significantly larger than the 2-Mu-M mean (Fig. 5.6c). The only variable component of these two combinations is that of maternal experience and it may be that the maternal experience factor is mediating the effect of the other two. It is possible that differences in the mother's behaviour, due to the amount of maternal experience she had received, were perceptible

to the pups only when the maternal stimuli were not masked by stimuli from the father and from a large number of littermates.

The open field 'inner compartment ambulation' mean for 7-Pr-F was significantly larger than the 2-Pr-F, 2-Pr-M, 2-Mu-M, 7-Mu-F and 7-Mu-M means (Fig. 5.6d). This is not only a measure of ambulation, in common with the last analysis, but is also a measure of the degree to which the mice were prepared to leave the outer wall of the apparatus. Inbred laboratory mice tend to remain close to the wall (Fredericson 1953). It is possible that, once again, the mother's behaviour was mediating the litter-size and the paternal presence effects. If so, then the primiparous mother's display of maternal behaviour when in the presence of both the father and a large litter, caused the offspring's higher inner compartment ambulation score. Reading (1966a) found that BALB/c mice developed the characteristics of their foster mother regardless of the foster mother's strain. It would therefore be expected that if the mother's behaviour was altered by the father's presence and the litter size, then there would be a consequent change in the offspring's behaviour.

No attempt will be made to draw a conclusion about the emotionality of the subjects from the open field results. The comments made in Chapter 3 indicate that great care must be exercised when interpreting rodent open field results. Whimbey & Denenberg (1967) maintained that both ambulation and defaecation scores should be considered when attempting to draw conclusions from open field performance. No significant differences between open field defaecation scores were found for the L x M x P results in my own experiment.

4. General Discussion

Litter-size, maternal experience and paternal presence are factors which affected certain aspects of mouse pup development during the pre-weaning period. Litter-size and paternal presence are of interest because of their main effects on pup behaviour. Maternal experience is important because it is a factor which interacted with litter-size and paternal presence to influence pup behaviour. It is possible that the maternal experience factor mediated some of the effects of the other two factors.

The development of the pups was monitored with 16 dependent variables. Of these, 13 proved suitable for discriminating between differences in pup behaviour and development caused by the three social factors. 'Drinking water' proved unsuitable because this activity was observed so infrequently. 'Proximity to the mother' and 'locomotion' scores were not significantly affected by the three factors.

Of the three social factors examined; litter-size, maternal experience and paternal presence; paternal presence emerged as a very important and most interesting influence on pre-weaning mouse pup behaviour. General observations of paternal behaviour, together with the results discussed in this chapter, prompted a more detailed study of paternal behaviour. Chapter 6 examines this next research stage in more detail and addresses the question of the mechanisms through which the father affects the behaviour of developing mouse pups. A more detailed discussion on the effects of paternal presence is also provided in Chapter 6.

5. Chapter Summary

1. Litter-size, maternal experience and paternal presence are three factors which affected mouse pup behavioural development.

2. Pups in small litters spent a larger proportion of time in close proximity to the father and head-lifting; spent a smaller proportion of time suckling and obtained larger day 30 body weights than pups reared in large litters.

3. Pups with a primiparous mother spent a larger proportion of time in close proximity to the father than pups with a multiparous mother.

4. Pups developing in the presence of the father spent a larger proportion of time head-lifting, rearing and eating solid food; obtained higher defaecation scores during open field tests and obtained higher day 30 body weights than pups without the father present.

5. Whereas litter-size and paternal presence were both well represented in the main effects, maternal experience is of interest because of its involvement in two-way and three-way interactions.

6. Interactions between the three factors were important determinants of resultant pup behaviour and development. Combinations which were of special interest were :

   (i) the 2-Mu group which was associated with smaller 'in nest' and 'proximity to littermate' scores and larger 'rearing' scores than other groups.

(ii) the 2-Pr-F group which was associated with larger 'sniffing' and 'grooming' scores and smaller open field 'inner compartment ambulation' scores than other groups.

(iii) the 7-Mu-M group which was associated with smaller 'sniffing', 'grooming' and open field 'inner compartment ambulation' scores than other groups.

7. It is possible that the small-litter size and father-present factors were responsible for accelerating development in mouse pups. In order to explain these effects, attention was given to thermal, olfactory and tactile stimulation mechanisms. The small-litter pups were more likely to be influenced by parental stimulation because they faced less competition for the attention of their parents, than pups in a large litter.

8. The maternal experience factor appeared to either mediate some of the other effects or to affect pup behaviour itself through the mediation of some of the other factors.

9. The role of the father appeared to be more important and more interesting than originally anticipated. Paternal experience may have influenced the maternal experience results. In addition to the extra barrage of stimuli received by pups in the presence of the father, the father also appeared to fulfil a strong caretaking role which may have enriched or provided a partial substitute for the mother's caretaking behaviour.

10. A father may affect pup behaviour and development, directly or through a maternal mediation route. The route of paternal influence is explored in more depth in Chapter 6.

Chapter Six

THE FATHER'S INFLUENCE ON DEVELOPING PUPS

Chapter 6.

THE FATHER'S INFLUENCE ON DEVELOPING PUPS

1. Introduction

Work reported in Chapter 5 showed that mouse pup development was affected by the presence of the father. The purpose of this stage of the project is to examine more carefully the caretaking role of the father and to investigate the route through which the father influences mouse pup development.

Work carried out on the caretaking role of adult male rodents was reviewed in Chapter 1. Adult male rodents frequently display caretaking behaviour similar to that of the female, including grooming, touching and retrieving pups, and nest construction (Spencer-Booth 1970).

In the laboratory mouse the father remains close to the mother and the pups during the pre-weaning period and consequently has the potential to affect the pups, either directly or through the mediation of changed maternal behaviour (Mugford & Nowell 1972). These two routes of influence are shown in Fig. 6.a.

An example of the direct influence route would be changed offspring behaviour caused by the father providing the pups with tactile stimulation by walking over them or by keeping the pups warm by remaining in the nest with them. The maternal mediation route could be exemplified by the father lessening the caretaking burden of the mother, with the consequence that the quality of her maternal care

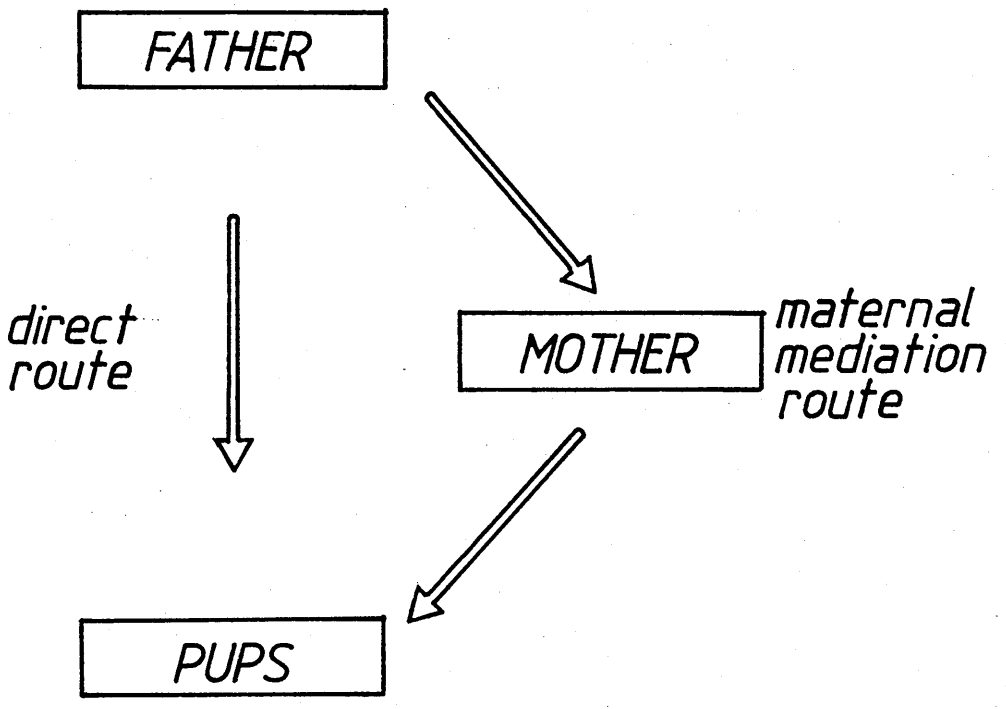


Fig.6.a Two possible routes of paternal influence on developing pups.

was improved and with this being reflected in the offspring development.

Two experiments were conducted to provide information on possible mechanisms by which the father influences pup behaviour. The first is called the 'Split Litter Experiment' and this investigated the role and the effect of the father on the pups in the absence of the mother. The design of this experiment prevented a maternal mediation of the father's effect on the offspring, whilst still permitting the father to directly influence the pups. The second experiment called the 'Split Cage Experiment', sought to elucidate the complex of inter-relationships between the father, the mother and the offspring. It created a situation whereby the pups could have access to the father and to the mother, without the two parents having access to each other.

Together, the two experiments tested the following two hypotheses:

1. The father directly influences pup behaviour and development.
2. The father influences pup behaviour and development through the mediation of changes in the mother's behaviour.

2. The Split Litter Experiment

Materials and Methods

Litters, consisting of nine pups, were split into three equal size groups and each group was subjected for part of the day to a particular parental influence. One group was placed with the father; another group with the mother; and the third group was housed alone, without access to either parent. This was repeated daily between days 10 and 29 inclusive. Offspring activities were recorded daily during the same period of time. Open field tests and body weight

measurements were conducted on day 30. Four replicates were used for this experiment.

The breeding schedule described in Chapter 2 was used to produce subjects. Housing of subjects and working conditions were also identical with those outlined in Chapter 2. Multiparous parents were used throughout in order to increase the probability of obtaining large litters. Larger litter sizes have been obtained with multiparous parents than with primiparous ones (Chapter 2).

On day 2 the litters were reduced in size, when necessary, so that they contained exactly nine pups. The mother, father and nine pups were then left undisturbed until day 9 when the father was removed and placed in a separate cage. From this point onwards the father was never returned to the cage containing the mother. Pups from each litter were separated into the three groups, for the first time, on day 10. Except for balancing out the sexes of the pups in the three groups, the pups in each litter were randomly allocated to one of the three categories and marked with a fur marker to enable recognition. Red and blue 'Agrimark' fur marker was obtained from Pfizer Ltd., Sandwich, Kent. From day 10 onwards the pups were subjected to their particular treatments for a certain length of time during the light phase of the day/night cycle.

Three different 'parental influence' environments existed in the form of three separate cages. Cage 1 contained the biological father of the pups; cage 2 contained no parents; and cage 3 was the original cage containing the mother (Fig. 6.b). Pups were transferred to their appropriate cages with the minimum of disturbance. Those pups which were to remain with the mother were, nevertheless, picked up,

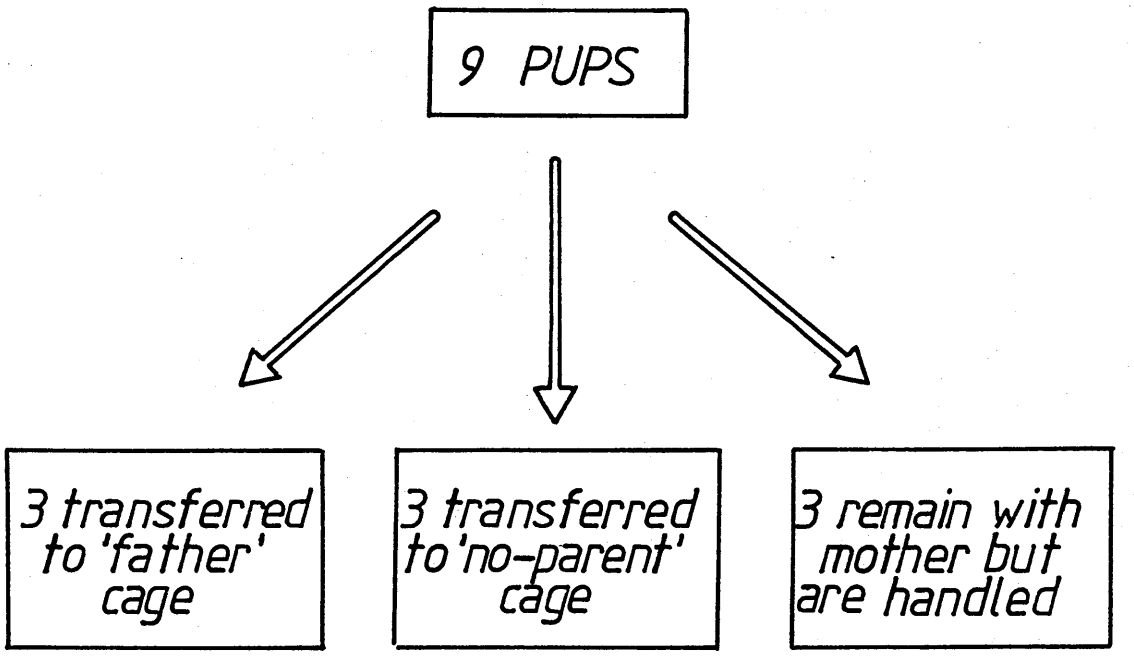


Fig. 6.b Division of litter into three treatment groups.

removed from the cage and replaced in order to simulate the treatment received by the other two groups whilst being transferred to their respective cages.

The length of time for which pups remained in their separate cages each day increased with pup age and was determined by a pilot experiment where the condition of the pups was very carefully monitored. Although experiments have already been conducted where BALB/c mouse offspring have been separated from their mothers (LaBarba & Hodge 1970; LaBarba, Lutz & White 1968; Newell 1967), it became necessary to identify the time period for which pups could be separated from their mother without causing them distress. It was obviously necessary to return all pups to the mother each day so that they could suckle. Food and water was made available in all three cages. Developing pups eat food pellet crumbs, which have dropped into the sawdust through adult feeding, before they feed from the food container incorporated in the cage lid. In order not to disadvantage pups in the 'no parent' cage, a food pellet was crumbled and particles placed in the sawdust, in anticipation of early pup ingestion of solid food.

Without incurring any apparent pup distress, the scale in Table 6.a was established and used with all of the litters in this experiment.

Table 6.a Duration of Treatment Period Each Day

Pup Age/Days	Separation Period Each Day
10	4 hours
11	5 hours
12	6 hours
13	7 hours
14-29	8 hours

An additional purpose of the pilot experiment was to establish how much contact with the pups the father would require in order to maintain his display of caretaking behaviour. It is conceivable that, were the father to lose his paternal state, antagonistic behaviour could be elicited and directed against the pups. Work has been conducted on the factors responsible for infanticidal behaviour in adult rodent males. Elwood (1977, 1980) found that naive gerbil males (Meriones unguiculatus) caged with non-pregnant females treated test pups as a source of food yet displayed paternal behaviour when caged with a pregnent female. Experienced males, however, never ate test pups. It is possible, therefore, that the gerbil fathers began to display paternal behaviour sometime before the first parturition and, that once initiated, it was maintained throughout the father's life.

Rosenberg & Sherman (1975) found that adult male rats killed pups, and this has also been observed in laboratory mice (Gandleman 1972, 1973a; Gandleman & Vom Saal 1975). Elwood (1983) has reviewed the literature on adult male rodent killing of pups and indicated that usually no killing of their own offspring occurred. This would suggest that the fathers could somehow discriminate between their own offspring and other pups.

It has been suggested that the presence of a pregnant female blocks the tendency for adult males to kill pups in gerbils (Elwood 1980) and wild house mice (Labov 1980). In my own Split Litter design, experienced males were used, but after day nine they had no access to adult females, pregnant or otherwise, and had only limited access to pups.

Although full details will be provided later on the behaviour displayed by the father and directed towards the pups, the father did not manifest any antagonistic behaviour towards the pups. The limited pup exposure did not, therefore, prevent the maintenance of paternal behaviour. Whether the same outcome would have resulted were the male to have been less experienced or naive, is worthy of consideration.

Each day, at the close of the separation period, all pups were returned to the cage containing the mother. The three pups already in this cage were again handled to simulate cage transfer. It was at this stage that identification marking was reinforced. Fig. 6.c shows the dorsal body positions used for marking the three categories of pups.

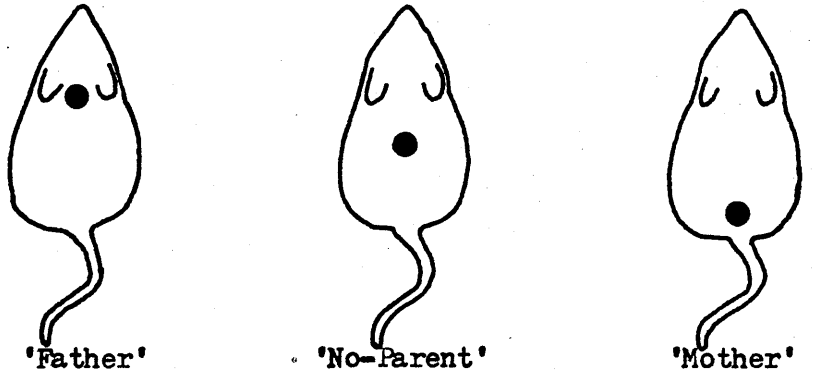


Fig. 6.c Dorsal Body Positions For Identification Marking Of Pups in the Split Litter Experiment

One pup from each category was selected to be the focal subject pup for observations and recordings of behaviour. Apart from equalizing the sexes throughout the three categories in the four replicate litters, focal pups were chosen randomly. Each focal pup received a blue identification mark, whereas the other two pups in each category received a red mark. Thus it was possible to recognize which pups belonged to each of the three categories and which pups were the focal pups. This ensured that each pup received the same treatment each day and that the same pups were used as subjects for observation each day. The design of the Split Litter experiment provided the opportunity to compare the effects of the pups' exposure to three different 'parental influence' environments, but ensured, nevertheless, that all pups were in sufficient contact with the mother to satisfy their physiological requirements. Any differences in pup behaviour that emerged under the three treatments must have resulted from differences in the behaviour of the mother compared with the father's, or either compared with no parent.

Daily Recording of Behaviour. One hour after the pups had been separated into the three cages, observations were made of their behaviour. All of this took place during the light phase of the day/night cycle. Initially, during a pilot investigation, observations were made under red light and made when the offspring were back together in the 'mother' cage. This practice of recording when the offspring were together was discontinued when it became apparent that any differences in offspring behaviour, due to the different treatments, were being masked by synchronized behaviour in the litter. This was particularly noticeable when the offspring were younger. Another disadvantage of recording the offsprings' behaviour when they were all back together with the mother was that the immediate effect

of the particular 'parental influence' treatment was not being recorded. By recording when the pups were in the three separate cages, additional information was provided on the relationships between the offspring and the parents. This meant that the roles of the father and the mother could also, to some extent, be compared.

Recording began on day 10 and continued daily until day 29. The same 12 activities were recorded as those listed for the main experiment in Chapter 3 (Table 3.2). Familiarity had been gained with the observation and recording of these activities and they were ideally suited for monitoring offspring behaviour in the Split Litter experiment. Suckling could only be measured, however, in the 'mother' cage; and proximity to the mother and proximity to the father could only be measured in the 'mother' cage and the 'father' cage respectively. Offspring were subjected to their particular 'parental influence' exposures up to day 29 in order to increase the likelihood that any treatment effects would be maintained up to day 30, when open field tests and body weight measurements were conducted. In comparison with the main experiment (Chapter 3), daily recording began at the same pup age but was continued for four extra days. Interest in activity trends past day 25 prompted the decision to continue recording right up to the day 30 tests.

With the daily recordings, all three cages were observed simultaneously and an instantaneous sampling technique was used with a time interval of 10 seconds between sound signals. This technique was discussed in Chapter 2. The behaviour of the pups, at the time of the signal, was recorded. The recording session was 16 minutes 40 seconds in length which represents 100 sets of results. The maximum score for any activity was therefore 100. In common with

the main experiment, the means of two successive days' results were calculated, providing 10 sets of results for each pup, for each activity.

Day 30 Measurements. On day 30, open field tests were administered on all nine members of each litter. Details of the test procedure and apparatus were the same as for the main experiment (Chapter 3) except that a longer period of time was used to sample each subject's behaviour, and additional activities were recorded. The test duration was eight minutes and the following activities were recorded:

Number of peripheral compartments entered

Number of inner compartments entered

Number of times subject reared up on hindlimbs

Latency to defaecation - a score of one, two or three was given, referring respectively to short (up to 1.5 minutes), medium (from 1.5 to 3.5 minutes), and long (above 3.5 minutes) duration.

Number of faecal boli produced by the subject

Latency to urination - scored in the same way as latency to defaecation

Number of times subject urinated

Rearing, latency to urination and urination have been proposed for use as reliable open field measures (Tachibana 1980). Once again, the open field test was included as an easy to administer test at the conclusion of the period of development being studied. It was considered likely that some of these open field measures would enable information to be gained about behavioural differences in the offspring, caused by the experimental treatments. Body weights of all offspring were also measured on day 30.

Data Treatment. The procedure adopted for the main experiment was again used with the Split Litter data. This procedure is described in Chapter 3 where reasons are provided for its adoption. Data obtained from the daily observations were transformed (ARCSIN) and subjected to analyses of variance and post-ANOVA Scheffé tests. Age was treated as a covariate which resulted in a one-way ANOVA with parental influence as the independent variable. Pearson product moment correlation analyses were administered between individual pup scores for behaviours under each separate experimental condition and pup age. From the 12 activities measured, it was possible to conduct nine analyses. The reduction from 12 to nine was due to the following. Proximity to the mother was compared with proximity to the father. Suckling was not subjected to the analysis because this was only applicable with the 'mother' cage. Drinking was also excluded on the grounds that it was exhibited too infrequently to make a meaningful comparison between the treatment groups. Analyses of variance were also administered to the untransformed litter means of the day 30 data.

Results

During the time that the offspring were in the three separate 'parental influence' cages, an opportunity was provided to examine the relationship between each of the two parents and their respective three pups. In addition to the objective data collected on the offspring behaviour during the formal recording sessions, more subjective observations were made of the behaviour of the mother and, especially, the father in response to the presence of the pups. A descriptive summary of these observations is provided before the analysis tables and graphs are presented. The description is similar in purpose to that provided by Horner & Taylor (1969) on paternal behaviour in the rat (*Rattus fuscipes*).

Behaviour of the Parents in Reaction to the Pups. During observation sessions, the mother displayed the normal range of caretaking behaviour; including nursing, grooming, licking and laying over the young. The mothers were also involved in maintaining the nest area. This maternal behaviour corresponded with that observed during the main experiment.

The caretaking behaviour displayed by the father exposed to the pups without the presence of the mother, however, was more noticeable than that observed before in a cage containing the mother, father and pups. This is in common with Hatton & Meyer's (1973) observation that when the mother was removed from a litter of cactus mice (Peromyscus eremicus), the amount of paternal caretaking behaviour greatly increased. Waring & Perper (1979) found a similar effect in gerbils. It has also been found that when adult male laboratory mice were housed in communal nests, it was the virgin females which tended to care for the pups; but when the males were housed alone with the pups, they then displayed caretaking behaviour (Gandelman, Pashke, Zarrow & Denenberg 1970; Lown 1980). It was suggested that the male caretaking behaviour was inhibited in the presence of adult females.

The following observations were made on the role of the father:

1. The father was involved in nest construction by creating depressions in the sawdust for the pups and by collecting wood shavings to the nest area. This behaviour was exhibited to a lesser degree in the pups' absence, but was markedly intensified in their presence.

2. The father walked on and over the pups repeatedly. This source of tactile stimulation was continued, sometimes for long periods of time, and was more than merely the random movements of the father around the cage. The father gravitated towards the pups and focussed his attention on that corner of the cage.

3. The father sat on the pups for long periods and frequently began displaying this behaviour as soon as the pups were placed in his cage.

4. The father lay over the pups in a manner characteristic of the mother during nursing episodes. During this behaviour, it was usual for the pups to make the high frequency sounds which are normally associated with nipple seeking. It is likely that these audible signals were also accompanied by ultrasounds as will be discussed below. Whilst the pups were under the father during the time he was adopting the 'nursing' position, they tunnelled around as if attempting to locate nipples.

5. During the times the offspring were in their nests there was more contact between the father and his pups than between the mother and her pups. This was not only noticeable with casual observations, but was also reflected in the objective 'proximity to parent' scores (Table 6.3 and Fig. 6.3).

6. The father licked the pups. This behaviour is normally associated with maternal behaviour, but was well represented in the father under these circumstances.

7. When the pups were out of the nest and moving around the cage, there was considerable contact between the father and the pups. The initiation of the contact came mainly from the father and this usually took the form of a series of touches with one forepaw as he moved around the cage.

8. When not in contact with the pups, the father was usually very close to them. The pups appeared to constitute an effective stimulus to the father, causing him to focus his attention on them for a large proportion of the time. Most of the father's behaviour appeared to revolve around the existence of the pups. For example, when self-grooming, the father would often perform this activity in the nest with the pups.

9. Very little pup retrieval was observed with either the mother or the father, but some retrieval was performed by the father. The amount of pup retrieval by the father was not exceeded by that of the mother. Whenever the father carried pups, this behaviour always resulted in pups being returned to the nest area.

10. Those pups remaining with the mother spent a large proportion of time suckling; an activity not possible in the other two 'parental influence' cages. Over the recording periods and across the four litters, the mean percentage of time spent suckling was 24.25 ± 6.43 (S.E.M.). This considerable period of time represents a large suckling deprivation for the pups in the 'father' and the 'no parent' cages.

Tables and Graphs. Summary tables for all the analyses are now presented. Table 6.A provides a summary of all the age analyses. It shows significant age effects for the measures, as revealed by the analysis of covariance. These are general age effects, across all three experimental treatments, for each behavioural activity. In addition to this, Table 6.A shows, with Pearson product moment correlation analyses, how the age effects are distributed between the different treatments. Tables 6.1 to 6.17 summarize the results of the analyses of variance. These show the sources of variance, the mean squares (MS), the degrees of freedom (df) and the F ratios. The probability levels are given when there are significant effects. The results of the Scheffé tests are given, where applicable. Any two means not underlined by the same line are significantly different. Any two means underlined by the same line are not significantly different (Duncan 1955). (Tables 6.1 to 6.9 are based on transformed data. In these tables, backtransformed means, expressed as percentages, are provided in parentheses.) Even when there is a significant effect with an activity which has only two levels of the 'parental influence' variable, the Scheffé test is still administered for reasons of consistency of procedure.

For the daily recordings, graphs are provided of the mean percentage of instantaneous scans of pups performing each activity, plotted against pup age (Figs. 6.1 to 6.9). Each number on the 'pup age' axis refers to the first day of the pair of days from which each result had been obtained. For example, '18' refers to the overall score obtained by averaging the two scores from days 18 and 19. Histograms of the day 30 results are also provided (Figs. 6.10 to 6.17). It should be noted that Fig. numbers correspond to Table numbers. For example, Fig. 6.1 is based on the same activity data as Table 6.1. The three treatments are represented by the symbols

Table 6.A Age Analyses For Split Litter Treatment Effects

Values for F, df, and P are shown for the Covariate analyses

Values for r (correlation coefficient) and P (two-tailed) are shown for the Pearson Correlation analyses

MEASURE & Covariate values		TREATMENTS & Correlation values F	NP	M
IN NEST F=208.598 df=1,116 P<0.001	r P	-0.818 <0.001	-0.812 <0.001	-0.706 <0.001
PROXIMITY TO LITTERMATE F=97.444 df=1,116 P<0.001	r P	-0.620 <0.001	-0.754 <0.001	-0.363 <0.05
PROXIMITY TO PARENT F=0.021 df=1,77 NS	r P	0.002 NS		-0.084 NS
LOCOMOTOR BEHAVIOUR F=0.393 df=1,116 NS	r P	0.014 NS	0.132 NS	-0.077 NS
SNIFFING F=2.222 df=1,116 P<0.01	r P	0.195 NS	0.285 NS	0.332 <0.05
HEAD-LIFTING F=4.726 df=1,116 P<0.05	r P	0.165 NS	0.334 <0.05	-0.017 NS
REARING F=11.701 df=1,116 P=0.001	r P	0.244 NS	0.276 NS	0.264 NS
GROOMING F=4.734 df=1,116 P<0.05	r P	0.251 NS	0.158 NS	0.263 NS
EATING SOLID FOOD F=132.889 df=1,116 P<0.001	r P	0.753 <0.001	0.786 <0.001	0.459 <0.01

F : father

NP : no parent

M : mother

'F', 'NP' and 'M'; which refer respectively to the 'father' influence, the 'no parent' influence and the 'mother' influence. All graphs are based on untransformed data.

The tables and graphs are preceded by a summary of all of the results, and are followed by a summary table (Table 6.b) showing the significant differences between the scores obtained from the three treatment groups. These differences are then considered in more detail in the discussion section which follows.

Summary of Results. Of the 17 analyses carried out, 7 involved significant differences. (The numbers accompanying the behaviour headings, below, match the Table and Fig. numbers.)

1. In Nest. There was no significant difference between the scores obtained from the three treatment groups (Table 6.1). The analysis of covariance revealed a significant age effect ($P < 0.001$) and the correlation analyses showed a significant negative correlation between this activity and age for all treatment conditions (Table 6.A). The pattern of change over the recording period was similar for all three groups of pups (Fig. 6.1).

2. Proximity to Littermate. There was no significant difference between the scores obtained from the three different groups (Table 6.2). The analysis of covariance revealed a significant age effect ($P < 0.001$) and the correlation analyses showed a significant negative correlation between this activity and age for all treatment conditions (Table 6.A). Once again the pattern of change over the recording period was similar for all treatment groups (Fig. 6.2).

3. Proximity to Parent. The F pups had a significantly greater

overall mean score than the M pups (Table 6.3). The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.A). The pattern of change over the recording period was similar for the two groups (Fig. 6.3).

4. Locomotor Behaviour. The F pups had a significantly greater overall mean score than the M pups (Table 6.4). The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.A).

5. Sniffing. The ANOVA indicated that there was a significant difference between the treatment scores, but the Scheffé test result did not show this difference (Table 6.5). A comparison of the mean scores showed that the F pups had the largest mean and the NP pups had the smallest. The analysis of covariance revealed a significant age effect ($P < 0.01$) and the correlation analyses showed that there was a significant positive correlation between the M scores and age for this activity only (Table 6.A). Fig. 6.5 shows that the display of sniffing in the F pups was particularly pronounced between days 18/19 and 24/25. These high scores are likely to have been primarily responsible for the absence of a significant correlation with age in the F scores. Since this period coincides with the time when the 'proximity to father' scores were also high (Fig. 6.3), the sniffing may well have been elicited by the father's presence and odours.

6. Head-Lifting. Both the F pups and the NP pups had a significantly greater mean score than the M pups (Table 6.6). The analysis of covariance revealed a significant age effect ($P < 0.05$) and the correlation analyses showed that there was a positive correlation between the NP scores and age for this activity only

(Table 6.A). No immediate rational explanation, for a significant correlation under one condition only, is apparent.

7. Rearing. There was no significant difference between the scores obtained from the three groups for this activity (Table 6.7). Although the analysis of covariance revealed a significant age effect ($P = 0.001$), the correlation analyses showed that there was no significant correlation between any of the separate treatment scores and age (Table 6.A).

8. Grooming. There was no significant difference between the scores obtained from the three groups (Table 6.8). Again the analysis of covariance revealed a significant age effect ($P<0.05$), but the correlation analyses showed that there was no significant correlation between any of the three separate treatment scores and age (Table 6.A).

9. Eating Solid Food. There was a significant difference between the treatment scores according to the ANOVA, although the Scheffé test did not show this difference (Table 6.9). A comparison of the mean scores showed that the NP pups had the largest mean and the M pups had the smallest. The analysis of covariance revealed a significant age effect ($P<0.001$) and the correlation analyses showed that for all treatment conditions there was a significant positive correlation between this activity and age (Table 6.A).

There was no significant difference between the scores obtained from the three treatment groups in:

10. Open Field, Peripheral Compartment Ambulation,

11. Open Field, Inner Compartment Ambulation,

12. Open Field, Rearing and

13. Open Field, Latency to Defaecation.

14. Open Field, Defaecation. The F pups had a significantly greater mean score than the M pups (Table 6.14).

There was no significant difference between the scores obtained from the three treatment groups in:

15. Open Field, Latency to Urination and

16. Open Field, Urination.

17. Body Weight. The M pups had a significantly greater mean score than the NP pups (Table 6.17).

Table 6.1 Analysis of Variance of 'In Nest' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.040	0.533	NS
RESIDUAL	116	0.075		

Table 6.2 Analysis of Variance of 'Proximity To Littermate' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.092	1.892	NS
RESIDUAL	116	0.049		

Table 6.3 Analysis of Variance of 'Proximity to Parent' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	1	0.791	4.471	<0.05
RESIDUAL	77	0.177		

Parental Influence :	M	F
Means :	0.554	0.753
	(27.68)	(46.76)

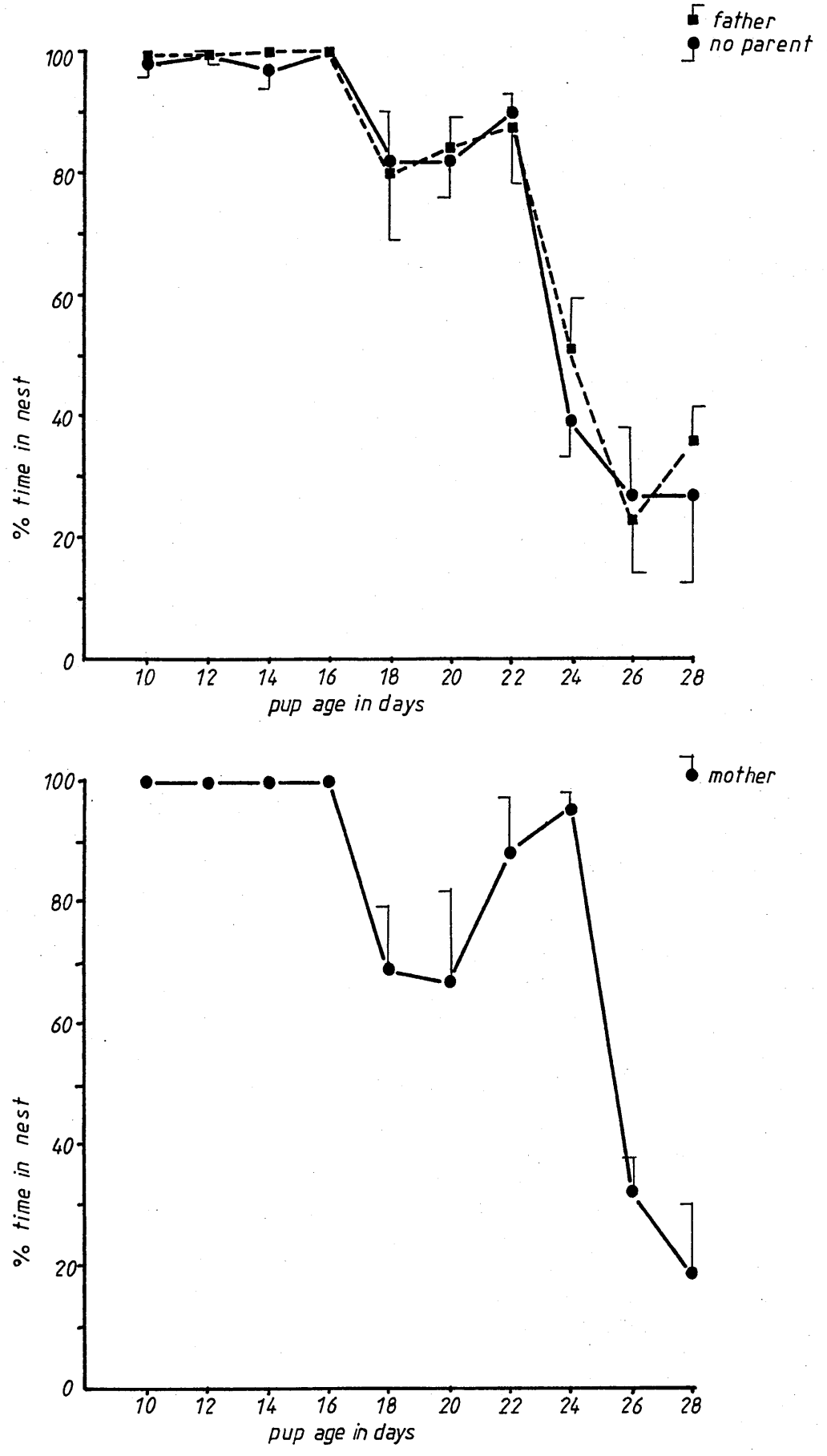


Fig. 6.1 *Mean percentage of instantaneous scans of pup in nest. Standard errors are indicated.*

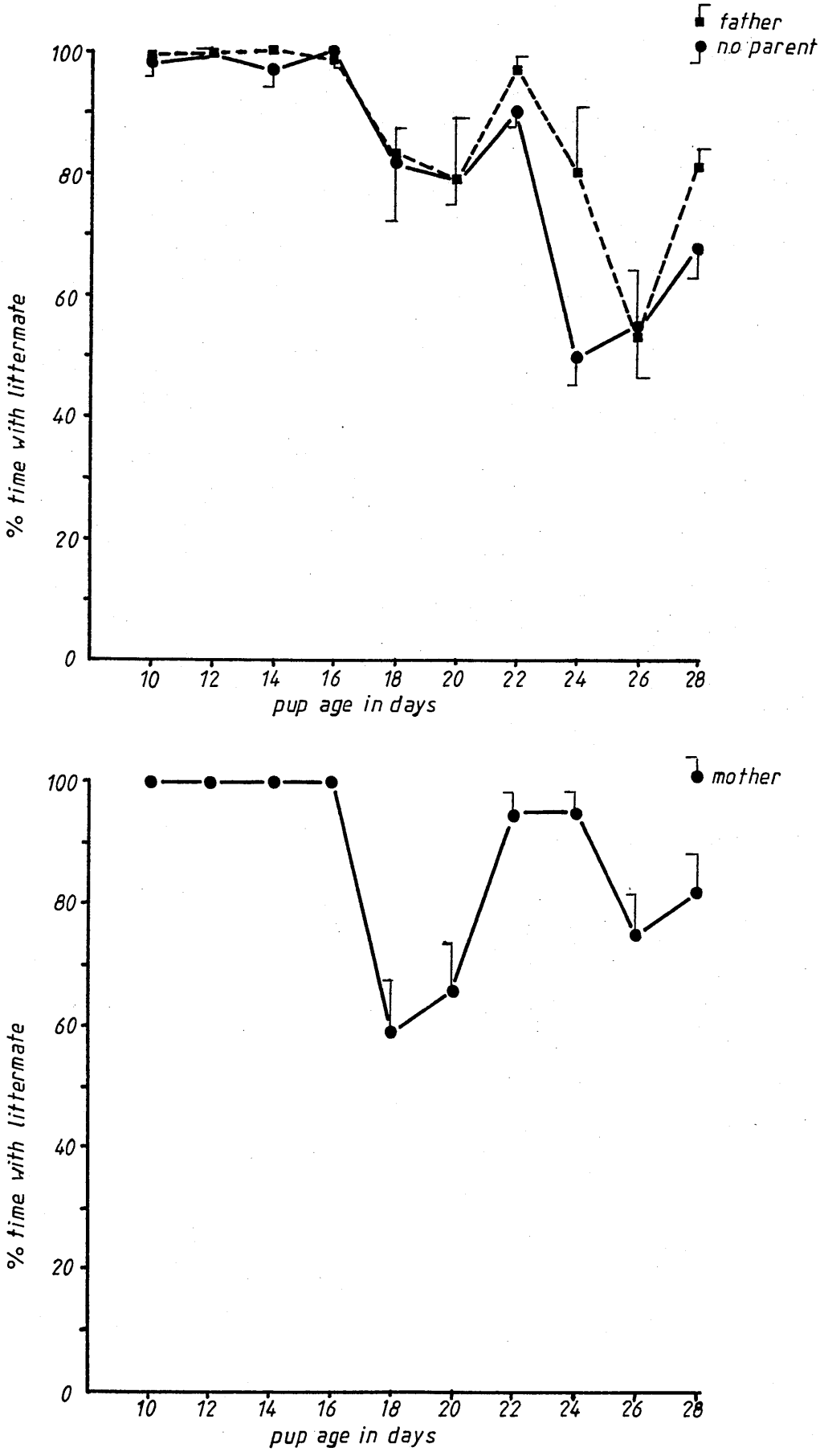


Fig.6.2 *Mean percentage of instantaneous scans of pup with littermate. Standard errors are indicated.*

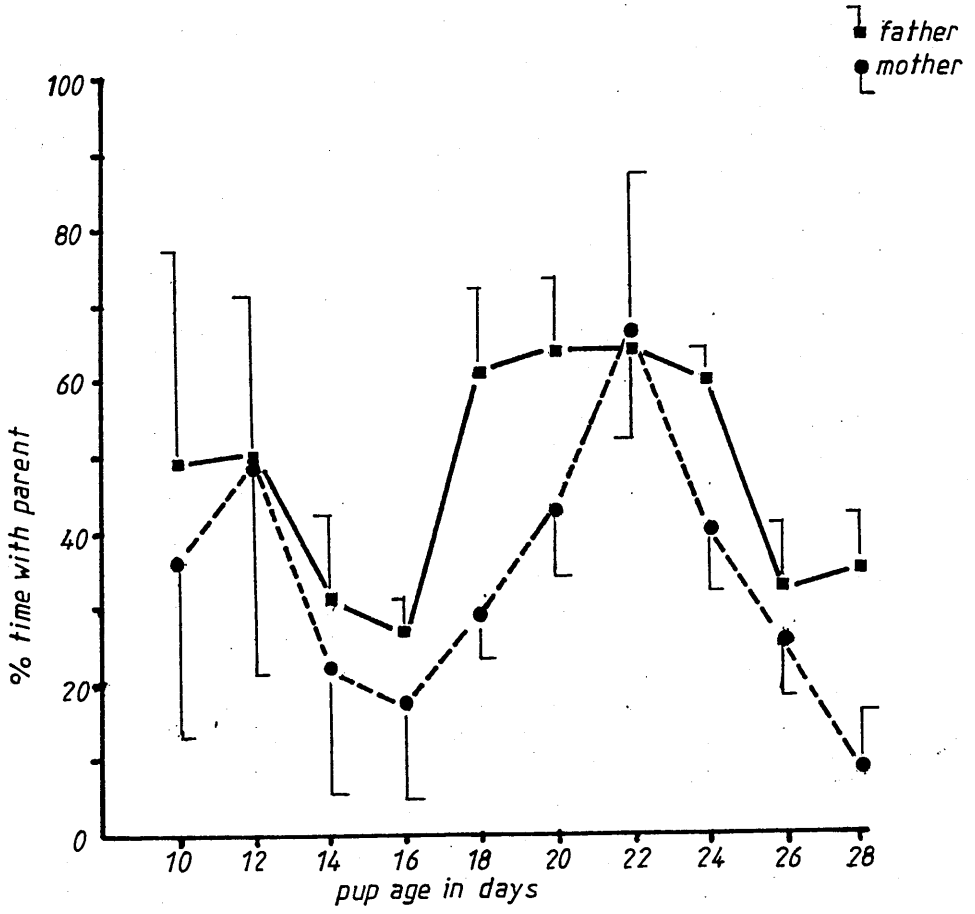


Fig. 6.3 *Mean percentage of instantaneous sampling scans of pup with parent. Standard errors are indicated*

Table 6.4 Analysis of Variance of 'Locomotor Behaviour' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.106	4.395	<0.05
RESIDUAL	116	0.024		

Parental Influence :	M	NP	F
Means :	0.249	0.271	0.347
	(6.07)	(7.17)	(11.56)

Table 6.5 Analysis of Variance of 'Sniffing' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.056	3.140	<0.05
RESIDUAL	116	0.018		

Parental Influence :	NP	M	F
Means :	0.079	0.104	0.153
	(0.62)	(1.08)	(2.32)

Table 6.6 Analysis of Variance of 'Head-Lifting' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.044	6.162	<0.01
RESIDUAL	116	0.007		

Parental Influence :	M	F	NP
Means :	0.023	0.075	0.085
	(0.05)	(0.56)	(0.72)

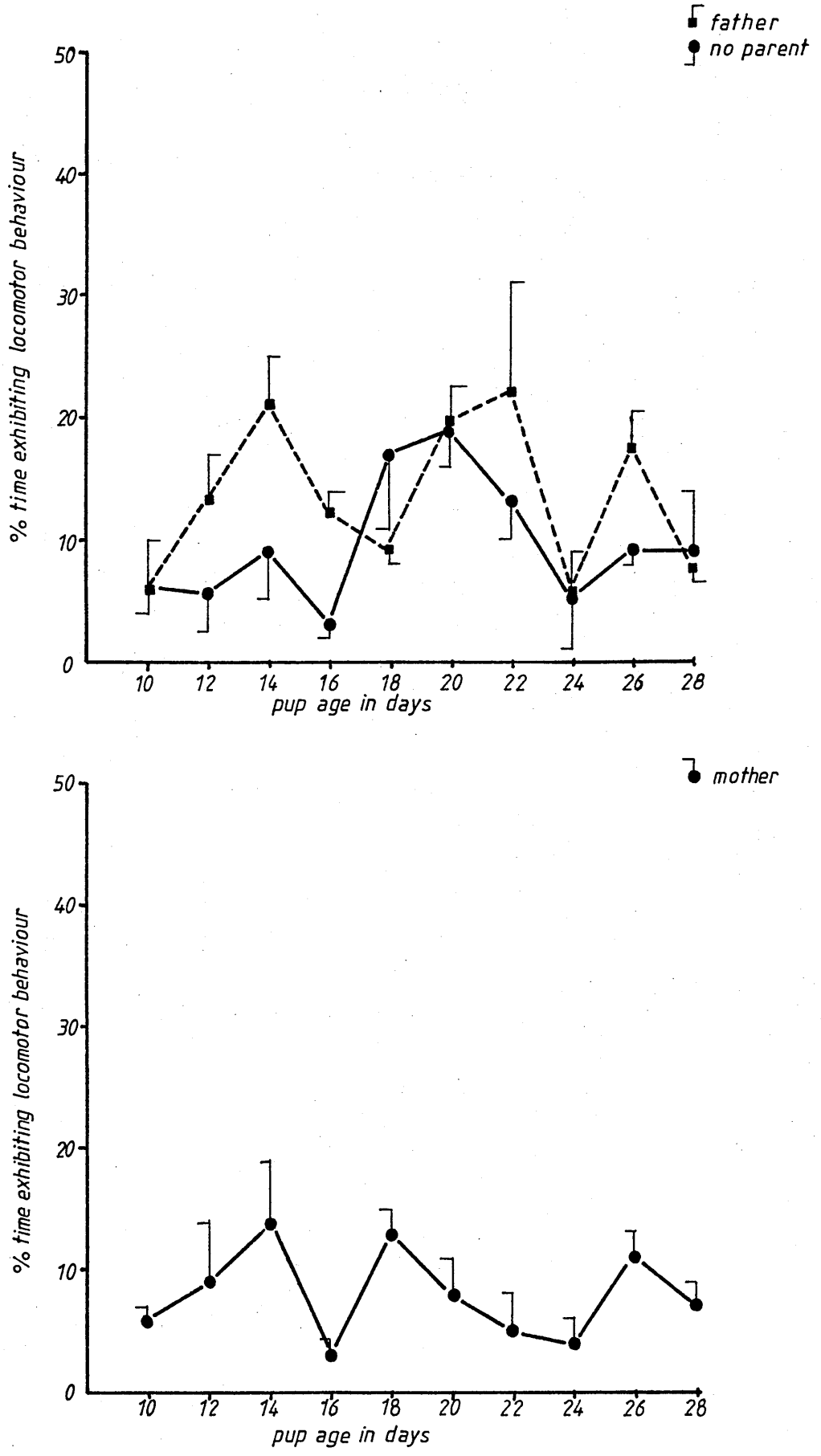


Fig. 6.4 *Mean percentage of instantaneous scans of pup exhibiting locomotor behaviour. Standard errors are indicated.*

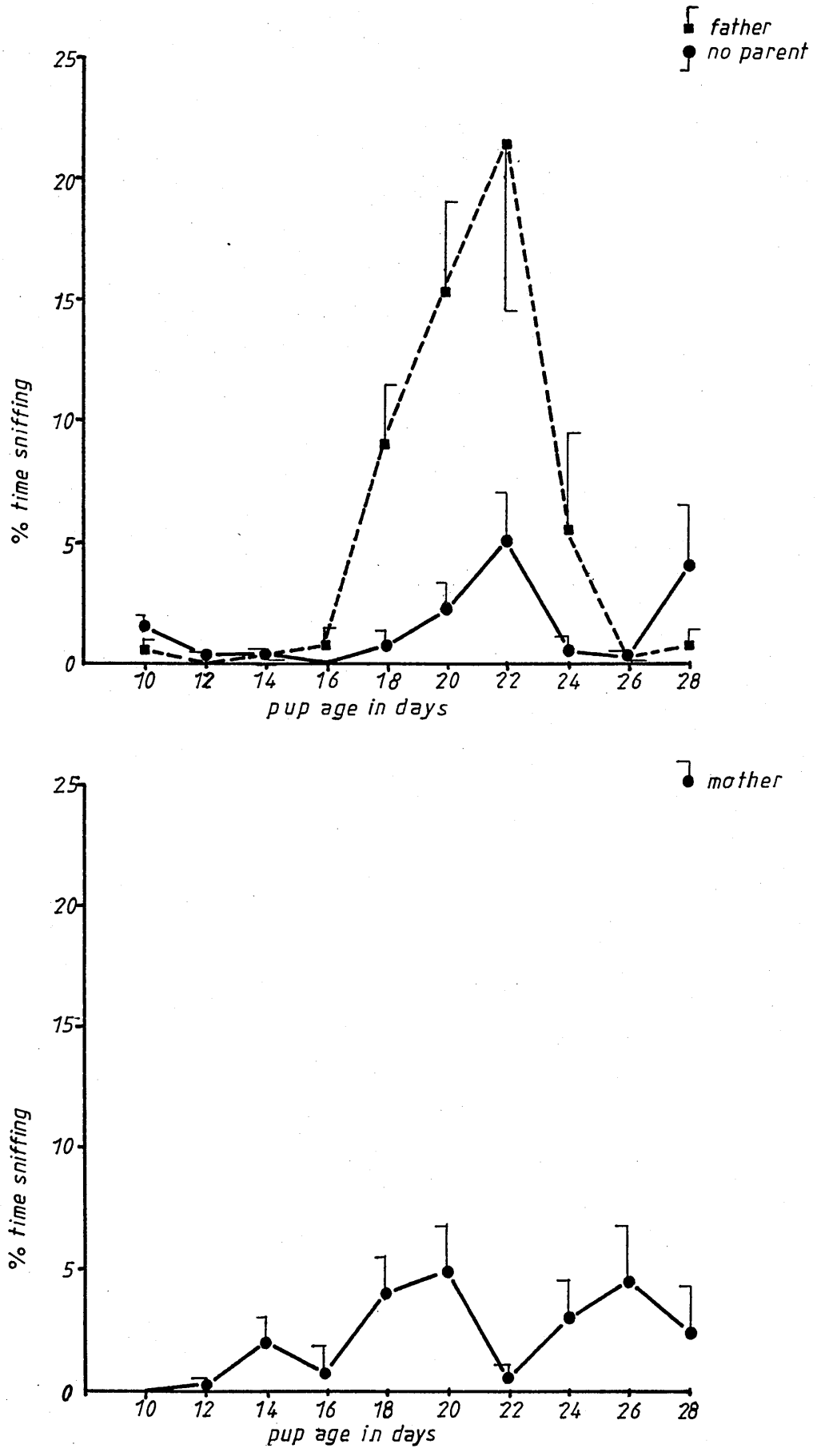


Fig. 6.5 *Mean percentage of instantaneous scans of pup sniffing. Standard errors are indicated.*

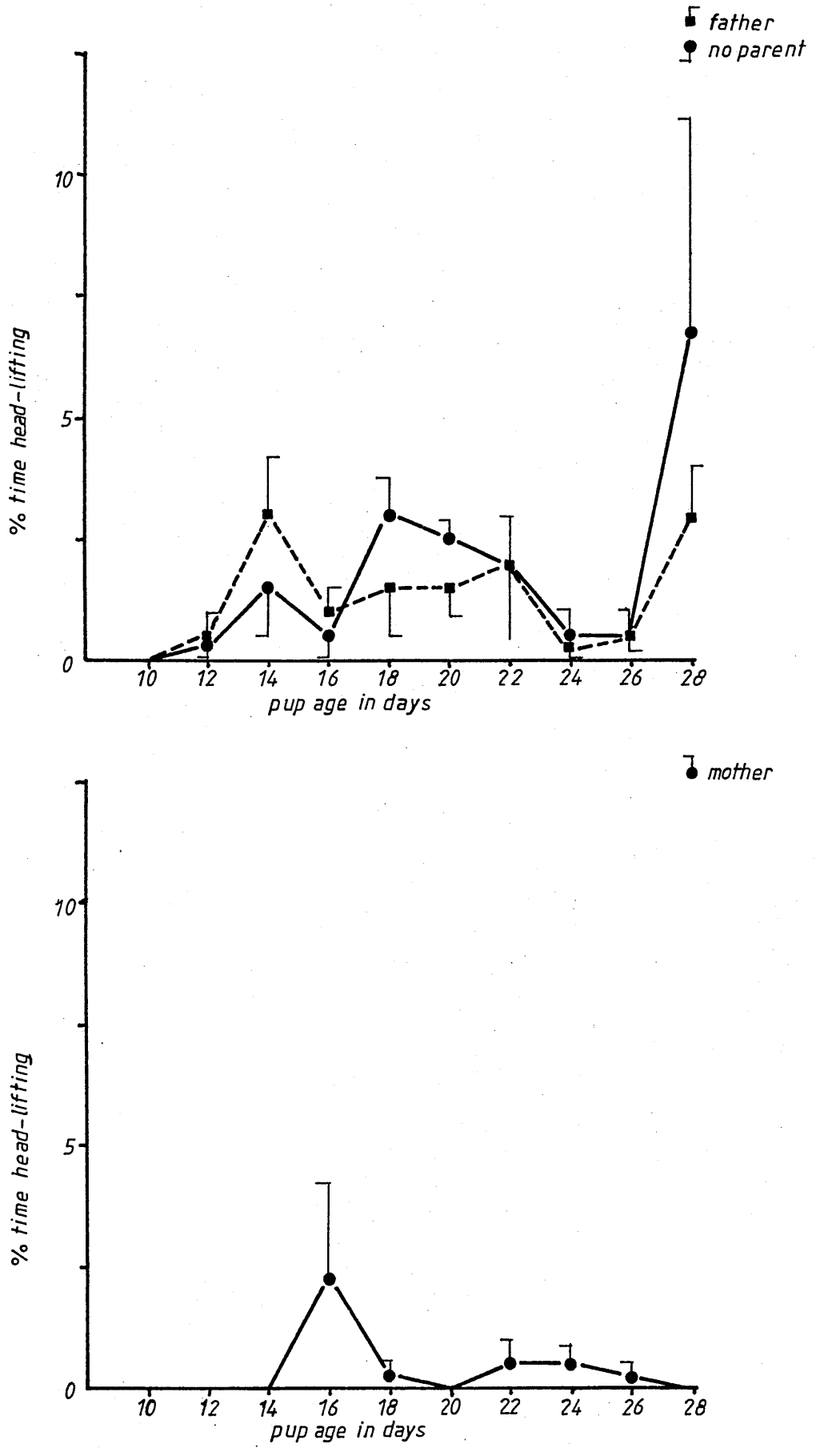


Fig. 6.6 *Mean percentage of instantaneous scans of pup head-lifting. Standard errors are indicated.*

Table 6.7 Analysis of Variance of 'Rearing' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.015	1.358	NS
RESIDUAL	116	0.011		

Table 6.8 Analysis of Variance of 'Grooming' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.023	0.634	NS
RESIDUAL	116	0.036		

Table 6.9 Analysis of Variance of 'Eating Solid Food' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.245	3.063	=0.05
RESIDUAL	116	0.080		

Parental Influence:	M	F	NP
Means :	0.188	0.308	0.335
	(3.49)	(9.19)	(10.81)

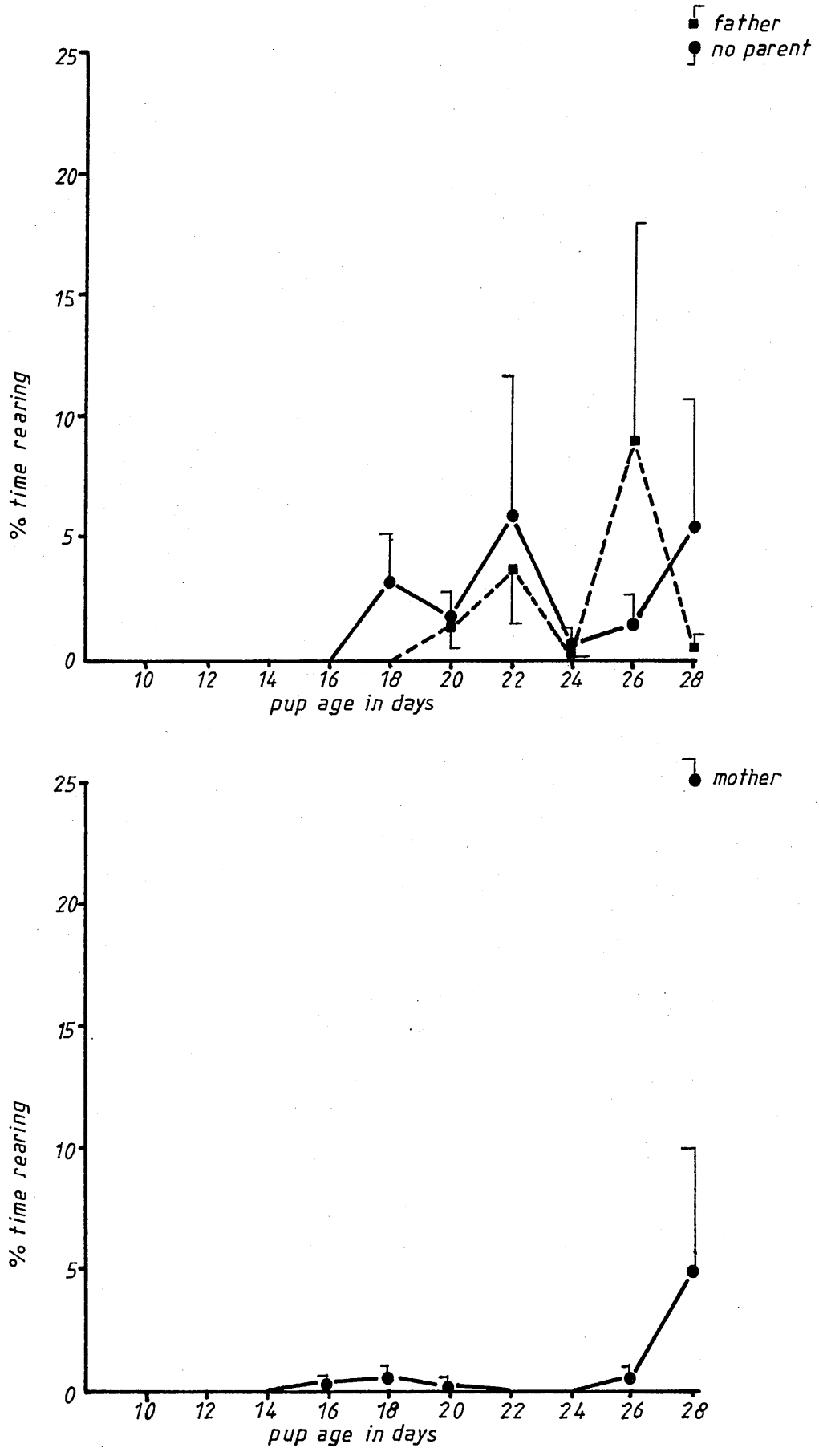


Fig. 6.7 Mean percentage of instantaneous scans of pup rearing. Standard errors are indicated.

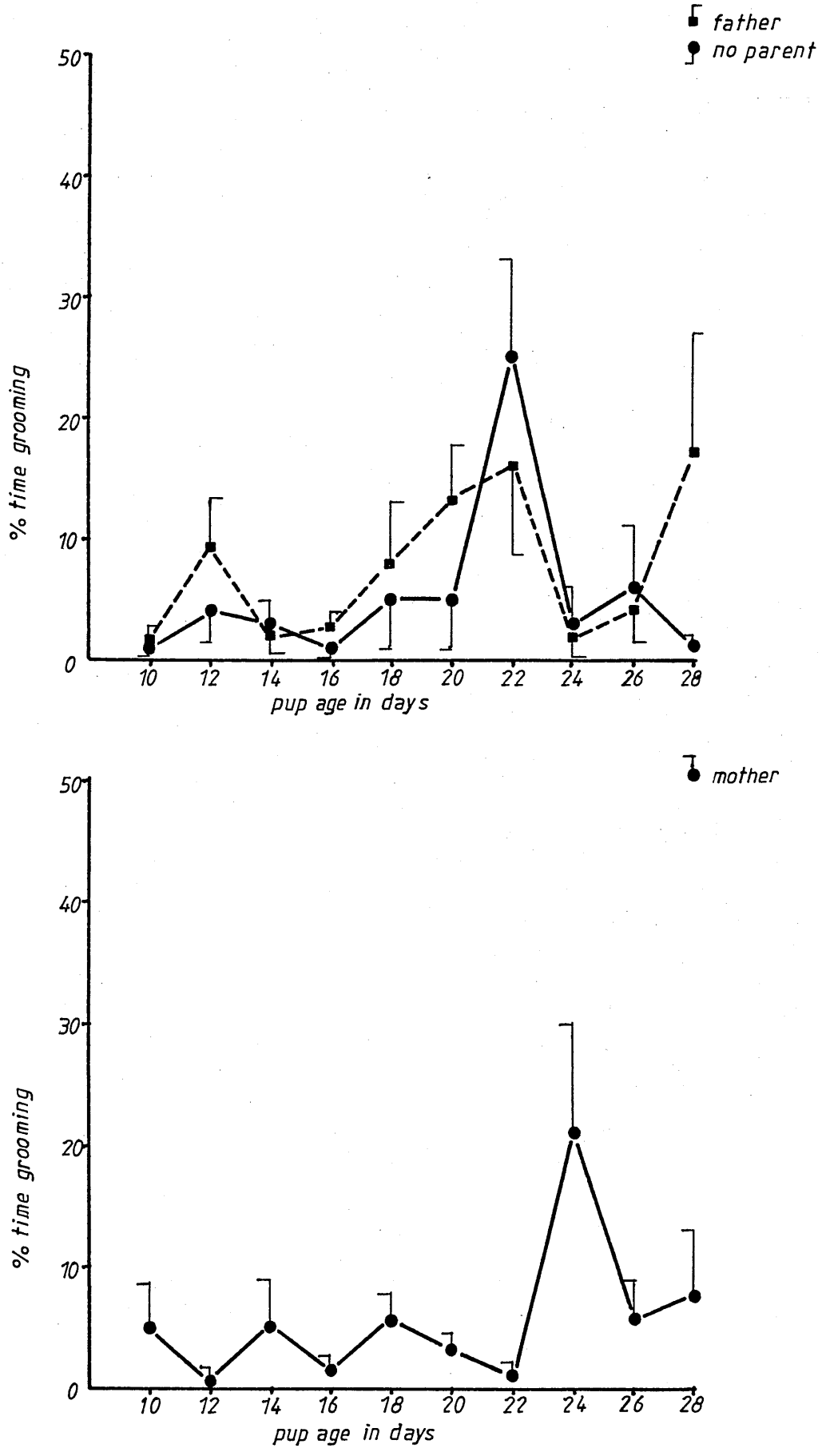


Fig. 6.8 *Mean percentage of instantaneous scans of pup grooming. Standard errors are indicated.*

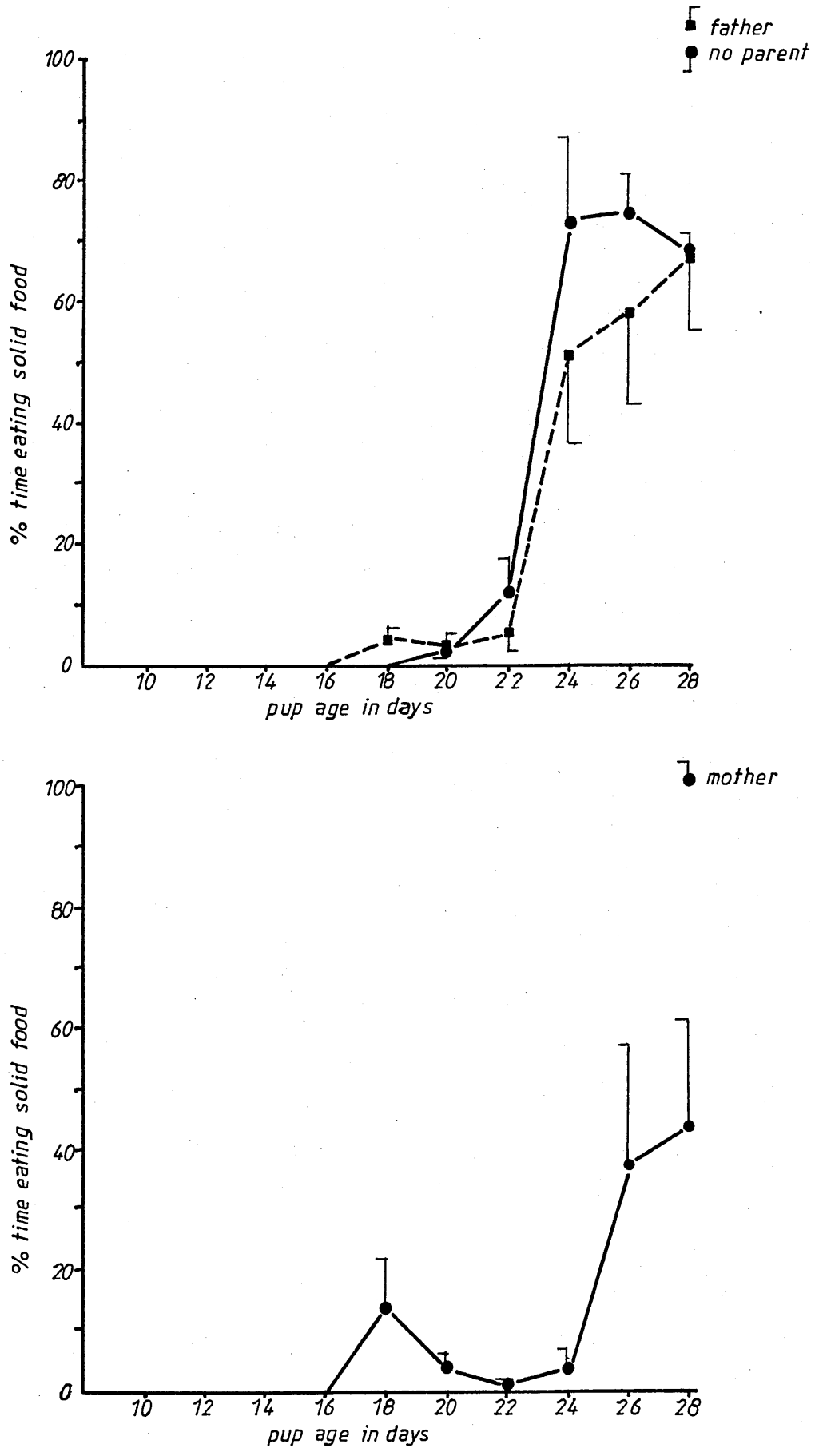


Fig. 6.9 *Mean percentage of instantaneous scans of pup eating solid food. Standard errors are indicated.*

Table 6.10 Analysis of Variance of Open Field 'Peripheral Compartment' Ambulation Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	2480.08	1.22	NS
RESIDUAL	9	2026.28		

Table 6.11 Analysis of Variance of Open Field 'Inner Compartment' Ambulation Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	306.25	0.35	NS
RESIDUAL	9	869.97		

Table 6.12 Analysis of Variance of Open Field 'Rearing' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	286.58	0.91	NS
RESIDUAL	9	311.97		

Table 6.13 Analysis of Variance of Open Field 'Latency to Defaecation' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.33	2.98	NS
RESIDUAL	9	0.11		

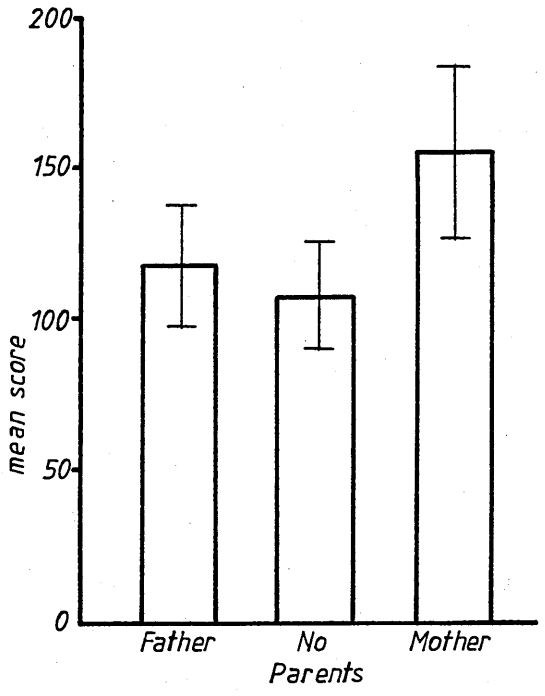


Fig. 6.10 Mean scores for open field peripheral compartment ambulation. Standard errors are indicated.

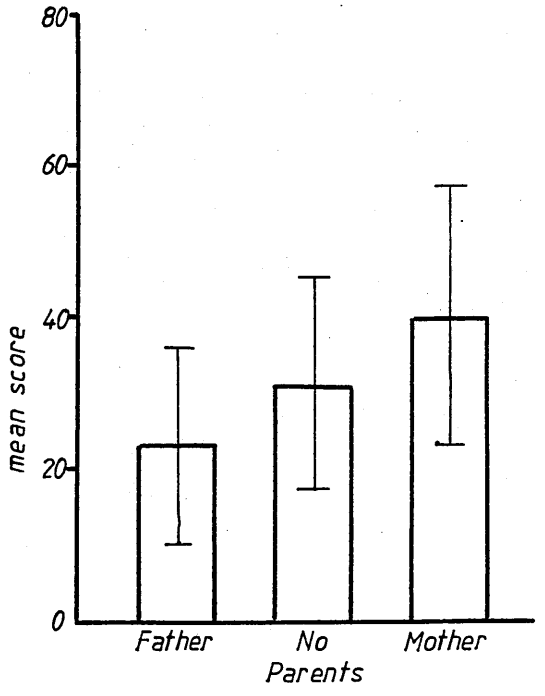


Fig. 6.11 Mean scores for open field inner compartment ambulation. Standard errors are indicated.

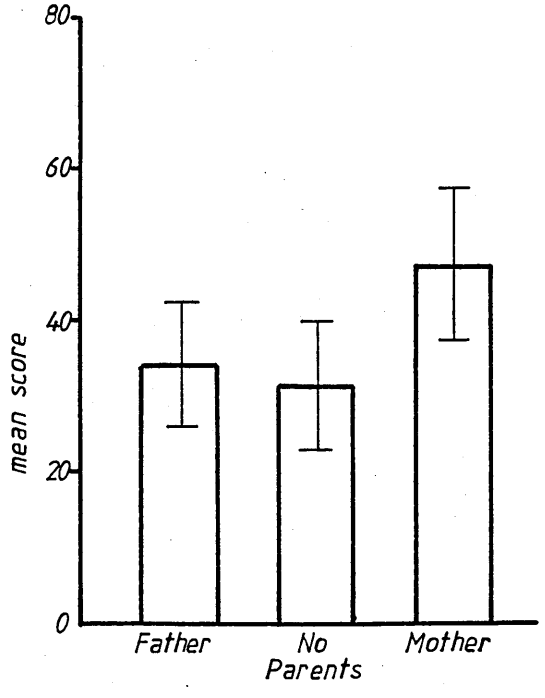


Fig. 6.12 Mean scores for open field rearing. Standard errors are indicated.

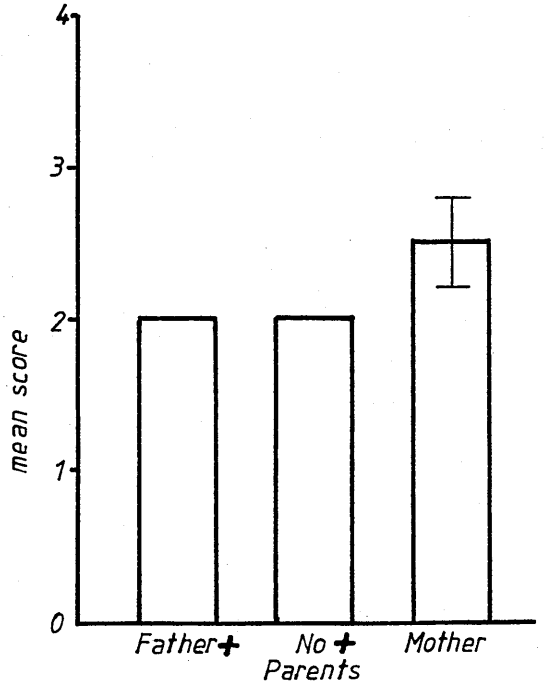


Fig. 6.13 Mean scores for open field latency to defaecation. Standard errors are indicated.

\+ Standard error = 0

Table 6.14 Analysis of Variance of Open Field 'Defaecation' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	6.08	5.76	<0.05
RESIDUAL	9	1.06		

Parental Influence:	M	NP	F
Means:	4	4.25	6.25

Table 6.15 Analysis of Variance of Open Field 'Latency to Urination' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.58	3.48	NS
RESIDUAL	9	0.17		

Table 6.16 Analysis of Variance of Open Field 'Urination' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	0.08	0.99	NS
RESIDUAL	9	0.08		

Table 6.17 Analysis of Variance of Body Weight Data with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Parental Influence	2	6.621	4.87	<0.05
RESIDUAL	9	1.360		

Parental Influence:	NP	F	M
Means:	8.89	10.88	11.29 *

* measurements in grams

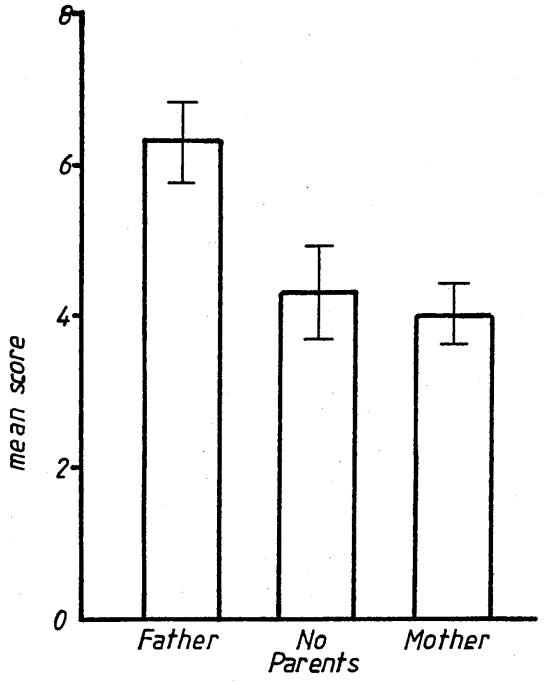


Fig. 6.14 Mean scores for open field defaecation.
Standard errors are indicated.

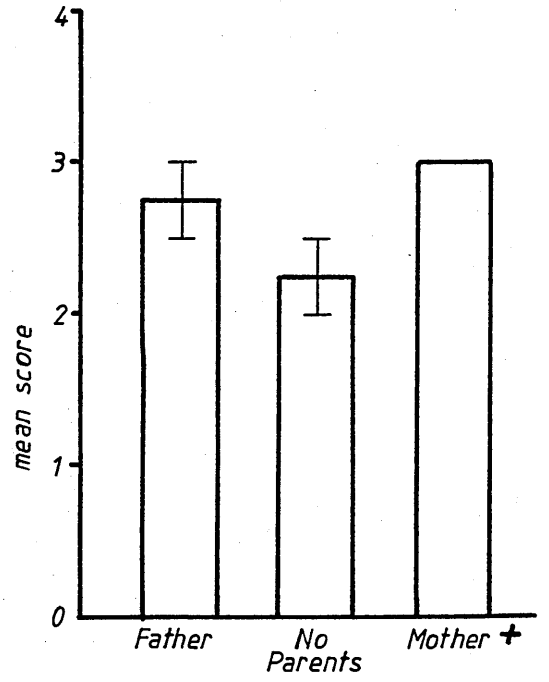


Fig. 6.15 Mean scores for open field latency to urination.
Standard errors are indicated.

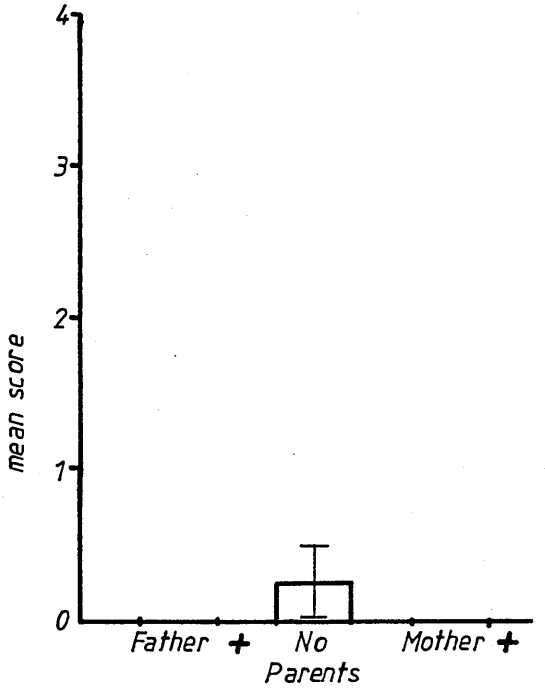


Fig. 6.16 Mean scores for open field urination.
Standard errors are indicated.

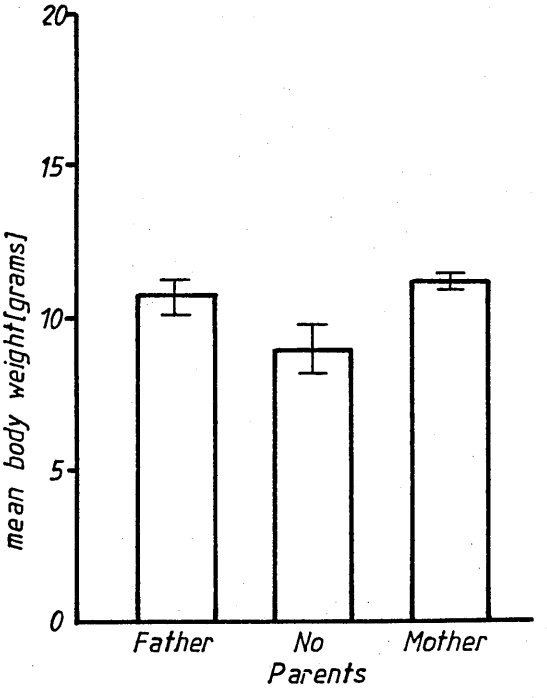


Fig. 6.17 Mean body weights [in grams].
Standard errors are indicated.

\+ Standard error = 0

Table 6.b

Summary of Significant Differences in the Results From the Split Litter Experiment

MEASURE	SIGNIFICANT DIFFERENCE		ANOVA P VALUE
Proximity to Parent	M	F	<0.05
Locomotion	M	F	<0.05
Sniffing	NP	F	<0.05
Head-Lifting	M	F	<0.01
	M	NP	<0.01
Eating Solid Food	M	NP	<0.05
Open Field Defaecation	M	F	<0.05
Body Weight	NP	M	<0.05

'F', 'NP' and 'M' refer respectively to the 'father' influence, the 'no parent' influence, and the 'mother' influence.

Discussion

The different 'parental influence' treatments caused significant differences in seven measures of pup behaviour and development. Five of these measures involved the father (Table 6.b).

The Father's Role. Offspring spent more time in close proximity to the father than to the mother (Table 6.3 and Fig. 6.3). As indicated in the early part of the results, this was principally caused by the father's exhibition of caretaking behaviour, rather than by the pups seeking to have contact with the father. With only one exception, on days 22/23, this effect continued over the entire recording period. It is possible that the father expressed more interest than the mother in the pups because they represented a more novel stimulus to him. Whereas the mother was always exposed to pups, the father was exposed to pups for no more than eight hours each day. Noirot (1964a, 1965) found that the tendency to perform some maternal responses was reduced by their performance in laboratory mice. It is also possible that, with a large litter size of nine pups, the mother was showing evidence of fatigue. The notion of maternal fatigue with large litters was suggested by Seitz (1958) who found that maternal behaviour in the rat varied inversely with the number of offspring in the litter. Two suggestions were made concerning this maternal fatigue theory. One was that a decline in maternal behaviour in large litters served to protect the mother from excessive fatigue, and the other was that fatigue induced in the mothers of large litters served to activate a stepwise reduction of maternal behaviour as the fatigue level rose.

Casual observations revealed that the father paid close attention to the pups and exhibited similar caretaking behaviour to that exhibited by the mother. Priestnall & Young (1978) reported that the laboratory mouse father displayed as much caretaking behaviour as the mother. It is likely that some characteristic of the offspring triggered this paternal response in the same way that maternal behaviour is released. Laboratory mice emit ultrasonic calls and these vary with the age of the pups (Noirot 1966). These calls are important for eliciting retrieval and nest building in adults. It is likely that when the mouse pups were transferred to the 'father' cage their body temperatures dropped and that this prompted the production of ultrasounds (Okon 1970a). It is also possible that the handling of the pups during their transfer to the three different cages caused the pups to produce ultrasounds which elicited parental care. Bell et al. (1971) studied this phenomenon in deermice (Peromyscus maniculatus bairdi) and found that mice handled during their pre-weaning period emitted ultrasonic sounds with higher mean peak frequencies and with longer mean durations than unhandled controls. Although it was suggested that these signals served to elicit increased maternal behaviour, it is conceivable that they could also release a similar effect in the male.

If ultrasounds emitted by the offspring were responsible for the caretaking response of the father, it is more likely that they were caused by a reduction of body temperature than by tactile stimulation. Noirot (1972b), in a review of rodent ultrasounds and paternal behaviour, put forward the idea that rodent pups in distress emit two different types of ultrasounds. One, caused by a response to cold, was seen to attract the adult to the pup. The other, caused by tactile stimulation, effected a withdrawal of the parent from the pup. It was suggested that the former type of ultrasonic signal would increase

as the pups increased in age and as homoiothermy developed, whereas the latter would show a decline after the first few days. It should be remembered that in my own experiment, pups were not transferred to the 'father' cage until day 10. Okon (1970b) further claimed that ultrasounds, induced in albino mouse pups by tactile stimulation, showed a sharp drop in intensity around day 6; four days before my own observations began.

In the Split Litter experiment, the father's close proximity to (Fig. 6.3), and interest in, the pups continued until day 29, when the daily observations were discontinued. Cold-induced ultrasound production may have been responsible for the earlier display of caretaking behaviour but it is likely that different mechanisms were responsible for its maintenance. Okon (1970a) found that these ultrasonic signals went into a decline from day 14 onwards and that there was complete cessation after day 20. Whereas the maintenance of caretaking behaviour may depend upon the suckling stimulus of the offspring in females (Porter & Doane 1978), a different mechanism must be proposed for adult males. Noirot (1969a,c) found that both auditory and olfactory stimulation from mouse pups primed different maternal responses and that a nursing position was adopted more readily in adults previously exposed to either type of stimulation. Naive animals only became fully maternal when they had become familiar with the odour of the pup (Noirot 1972a). Harper (1971) suggested that the appearance and behaviour of the young could facilitate caretaking by 'sensitizing' adults. A sensitized animal would be more likely to respond to offspring with caretaking behaviour. Reisbick et al. (1975) sensitized virgin femalerats by exposing them to pups. Rosenblatt (1967) reported that male rats would crouch over pups and lick pups provided there had been a long enough previous exposure to pups. Rat males which ignored pups on a first encounter developed parental

behaviour after continuous exposure to the pups (Wiesner & Sheard 1933). The sensitizing process in adults, due to exposure to pups, does not seem to depend on endocrinal changes. Leblond (1940) found that in male and female mice, even after eliminating gonad hormones by castration and hypophysectomy, caretaking behaviour could be evoked by exposing them to pups. The conclusion was drawn that nervous mechanisms were mainly responsible for the initiation and maintenance of caretaking behaviour. Lisk et al. (1969), however, found that introducing progesterone to adult male mice increased their tendency to build nests. This suggests that some parental activities are influenced by endocrinal mechanisms. Elwood (1979a) concluded that the causal factors for paternal behaviour in gerbils were the same as those responsible for maternal behaviour. It would appear that the Split Litter fathers had been sensitized by their previous exposure to the offspring and that continued exposure throughout the experimental period was sufficient for the maintenance of their display of caretaking behaviour. It is likely that the father was able to perceive the pups' ultrasounds, odours and general behaviour. Visual, tactual and gustatory sensory routes may well have been used also (Beach & Jaynes 1956b; Chantrey & Jenkins 1982).

Body Weights. When day 30 body weight measurements were taken, the M pups had a significantly higher mean body mass than the NP pups (Table 6.17 and Fig. 6.17). During the separation period for the pups each day, only the M pups would be able to suckle. Since they were doing this, on average, for nearly 25 per cent of the total observation time, it would be surprising if this difference in opportunity to suckle was not reflected in the body weights of the mice. Temporary suckling deprivation in the NP pups may also have been responsible for the significantly higher incidence of eating solid food in the NP group compared to the M group (Table 6.9). It may be that from day 24

(Fig. 6.9), suckling and eating solid food were interchangeable means of feeding, enabling a pups deprived of one to make more use of the other. Apparently, the NP pups' increased eating of solid food was not sufficient to compensate for earlier decreased suckling; hence their lower body masses.

The F pups were also deprived of suckling opportunities during their sessions with the father; and it would be expected that this deprivation would be reflected in smaller body masses compared to the M pups, yet there was no significant difference between their mean body mass and that of the M pups (Table 6.17 and Fig. 6.17). Further, the F pups did not show significantly inflated 'eating solid food' scores in comparison with the M pups (Table 6.9). Plaut & Davis (1972) deprived rat pups of the opportunity to suckle from day 13 onwards. Of those pups which had access to non-lactating parents there was less than a 20 per cent mortality rate compared to an 80 per cent mortality rate for those housed without an adult. A factor other than the nutritive one must be sought and the explanation could possibly involve thermal mechanisms. Both F and M pups were able to benefit from the thermal insulation provided by parental contact. The NP pups, consequently, would lose more body heat than each of the other two groups. This would mean that in the NP pups, once homoiothermy had developed, a larger proportion of the ingested food would be directed towards body temperature maintenance and away from the growth of body tissues than in the other groups. Okon (1970a) indicated that rodent pups develop homoiothermy during the first 19 days of their life, but that by day 15 they have developed homoiothermy at around 22^{o}C (laboratory temperature for this experiment). Chew & Spencer (1967) noted that rodent pups experience an elevated metabolic rate when exposed to low environmental temperatures. It has been claimed that the major contribution of the father in California mice

(Peromyscus californicus) (Dudley 1974a) and gerbils (Elwood & Broom 1978) is a thermal one. Dudley (1974a) found that when the mother was removed for 12 hours each day, pups developed at a faster rate if the father was caged with them. The father also played an important thermal role in my own experiment where he was involved in both nest building and remaining in contact with the pups in the nest; two activities likely to minimize temperature loss.

Other Offspring Activities Affected by Differing Parental Influence. Pups in the F group exhibited a significantly greater amount of locomotor behaviour than the M group pups (Table 6.4 and Fig. 6.4). Since locomotor behaviour was unaffected by the presence of the father in the main experiment, its appearance in the Split Litter experiment is likely to be associated with the fact that the pups were present with the father in the absence of the mother. Increased locomotor behaviour in the F pups may have been partly due to increased pup movement whilst attempting to locate nipples on the father, especially in the early part of the recording period. This would not provide an explanation, however, for elevated locomotor scores for the F pups throughout the entire recording period (Fig. 6.4). Schapiro & Salas (1970) found that 2 to 12 day old infant rats exhibited decreased activity levels when exposed to maternal odour. The maternal odour was more effective in inhibiting the activity of older pups than of younger pups. It is possible that in the Split Litter experiment, pups present with the mother showed a decreased activity level through this olfactory mechanism.

The F pups obtained high sniffing and head-lifting scores when compared to the other groups (Tables 6.5 and 6.6, and Figs. 6.5 and 6.6). For sniffing, the F mean was significantly greater than the NP means; and for head-lifting, the F mean was significantly greater than the M mean. Differences in these exploratory activities are quite consistent with the results obtained from the main experiment; where increased head-lifting and rearing were associated with the father's presence (Chapter 5); and may have been caused by the pups' responses to odours produced by the father.

For head-lifting, the M mean was not only significantly smaller than the F mean, but was also significantly smaller than the NP mean. Decreased head-lifting scores for the M pups may again represent inhibitions caused by maternal odour or may, instead, reflect an absence of mother-seeking behaviour. Since the mother is needed to satisfy the suckling need, it is possible that increased head-lifting was an investigative activity prompted by the absence of the mother.

A comparison of the open field defaecation measures revealed that the F mean was significantly greater than the M mean (Table 6.14 and Fig 6.14). This finding agrees with the main experiment result that pups raised in the presence of the father obtained a significantly greater mean defaecation score than pups raised in the father's absence (Chapter 5). It may be that a physiological or behavioural change was effected in the pups exposed to the father during their development and that this change was reflected in the open field defaecation scores.

Conclusions

The three different treatments in the Split Litter experiment had different effects on mouse pups' developmental behaviour. Housing the offspring for periods of time with the father only, revealed the strong caretaking role of the father, and the relationship existing between maternally deprived pups and the father.

In the main experiment, paternal presence caused a number of changes in the offspring's behaviour and development which are consistent with the results of the Split Litter experiment; especially those involving exploratory behaviour, open field defaecation and body weight. In the main experiment it appeared that development was accelerated in the presence of the father, but the experimental design did not allow the route of paternal influence to be established. Two routes were proposed: the direct route and the maternal mediation route. The Split Litter experiment has demonstrated that the laboratory mouse fathers were directly influencing the behavioural development of the offspring. Mouse pups, exposed to the father, in the absence of the mother experienced a close relationship with the father. Pups exposed to the father produced significantly larger mean values for 'proximity to parent', 'locomotion', 'sniffing', 'rearing' and 'open field defaecation' measures. Further, there was no expected significant depression of F pups' body weights, compared to the M pups. Now that the direct influence route has been substantiated, it remains to investigate the possibility of the existence of the maternal mediation route. The purpose of the Split Cage experiment is to provide information on this possibility.

3. The Split Cage Experiment

The Split Cage experiment investigated the paternal influence on the pups by both the direct and the maternal mediation route. The purpose of the experiment, however, was to establish whether maternal mediation really occurred.

It would be expected that if the presence of the father was affecting offspring behaviour and development through a maternal mediation effect, the mother's behaviour would also be changed due to the presence of the father. In order to ascertain whether any such change in maternal behaviour does take place, observations were made of both the offspring's and the parents' behaviour during the pre-weaning period of mouse pups.

Materials and Methods.

Central to the design of the Split Cage experiment is the actual cage. With this cage three different experimental conditions, concerning the father, were possible. The three conditions were as follows:

1. The father was housed with the mother and developing pups, where he could influence the pups either directly or through the mediation of changed maternal behaviour.

2. The father was housed in a different cage compartment from the mother, thus preventing physical contact between the two parents but allowing the pups, once mobile, to have access to either parent. This situation enabled the father to directly influence the pups, but

prevented or limited his opportunities to affect maternal behaviour. Under these circumstances, if the mother was to be influenced by the father, the nature of the influence would have to be auditory or olfactory.

3. The father was totally removed so that no type of paternal influence could affect the developmental behaviour of the offspring. The results from this condition provided a standard with which to compare the results obtained from conditions 1 and 2.

If the father was affecting the pups through only a direct route, the behaviour and development of the pups from conditions 1 and 2 would be expected to be similar; since the pups had access to the father in both conditions. If, however, only a maternal mediation effect was operating, the results from condition 1 would be expected to be different to those in the other two conditions. If the mothers' behaviour was similar in conditions 1 and 2, but different from condition 3, it would suggest that the father was influencing the mother through auditory and olfactory means.

The Split Cage. The design and dimensions of the cage are shown in Fig. 6.d. The cage unit described below is the end result of a long series of exploratory trials into the suitability of different cage designs. In order to carry out this work, four identical cages were made. No commercially produced cage was adequate to meet the requirements of this experiment. The principal feature of the cage is the aluminium 'pup filter' divider. Aluminium proved to be a satisfactory material because the mice were unable to enlarge the pup filter holes. Gnawing of holes occurred in earlier hardboard dividers. Pups up to the age of 30 days can crawl through the filter holes but adults are unable to do so. The size and shape of the filter holes

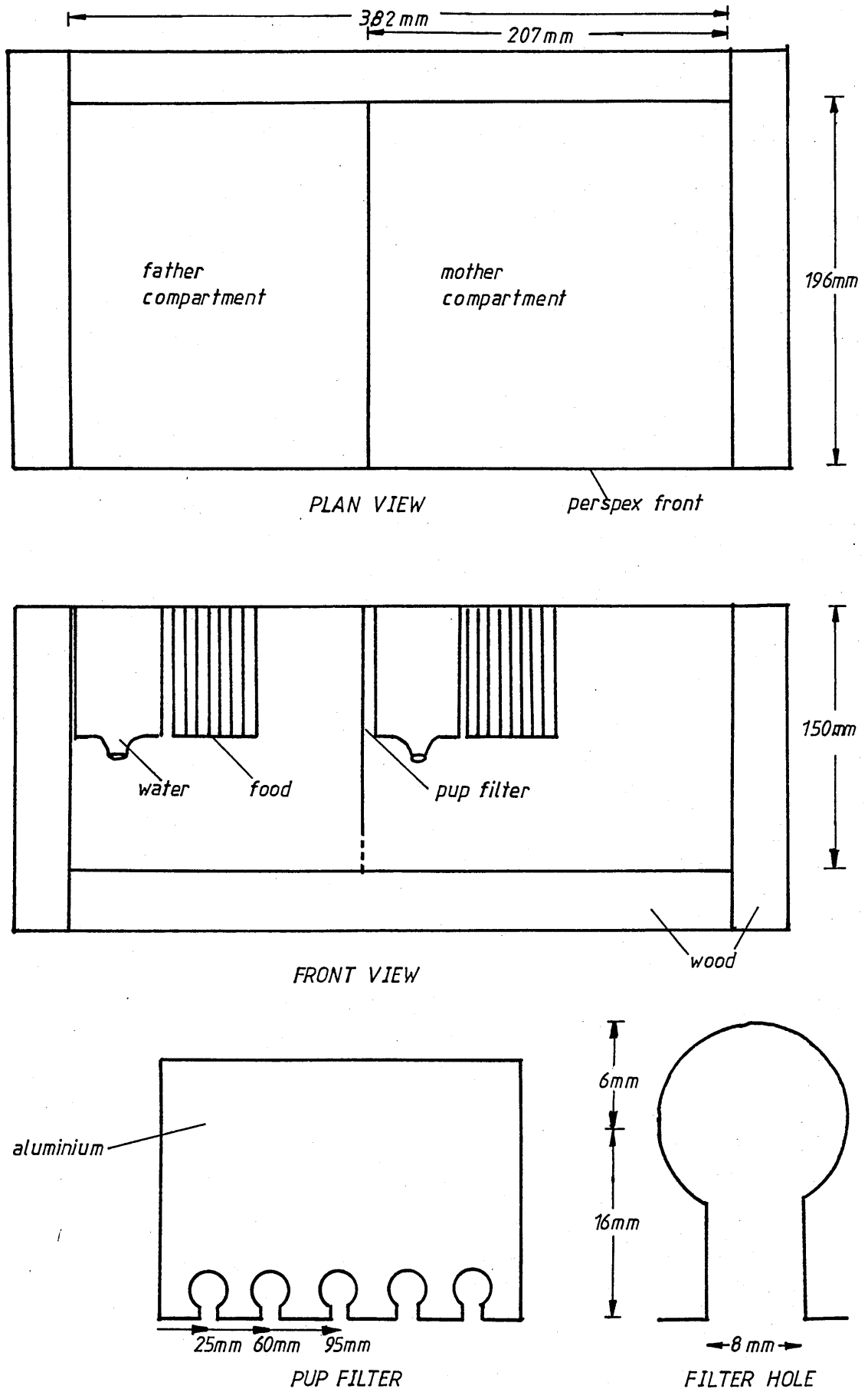


Fig. 6.d The Split Cage.

were determined by trial and error. I found that a slightly larger hole would not prevent adult movement through the filter, and a slightly smaller one was too small to allow older pups to move through. Many unsuccessful attempts to identify the appropriate hole size were made before these successful dimensions were arrived at. The position of this divider creates two compartments. The mother compartment is on the right hand side and it is here that the mother always stays. On the left hand side is the father compartment and when the father is placed here both parents were unlikely to see each other or to have physical contact with each other. (I considered that randomization of position of the compartments was unnecessary since being on the left or right side per se would not be expected to affect the subjects behaviour.) As soon as the pups were able to leave the nest in the mother compartment, they could have access to the father compartment through the pup filter holes. Figs. 6.e to 6.j show some views of the Split Cage apparatus.

The back and side walls, and the floor of the cage, were made of wood. The floor of the cage was lined with a piece of painted hard-board to prevent moisture soaking into the wood. The entire front of the cage consisted of perspex to enable total visual access to the occupants. The lid of the cage also made it possible to see the interior. Both the father compartment and the mother compartment had their own food and water dispensers made of stainless steel. Since these dispensers were made of parallel wire bars, they provided adequate ventilation for the cages. The rest of the lid was composed of perspex. Food and water were provided ad libitum. As described in Chapter 2, 'Production Mouse Diet' nuts were used for food. Softwood sawdust was added to the cages to a depth of 25 mm, with wood shavings to serve as nesting material. Environmental conditions were identical

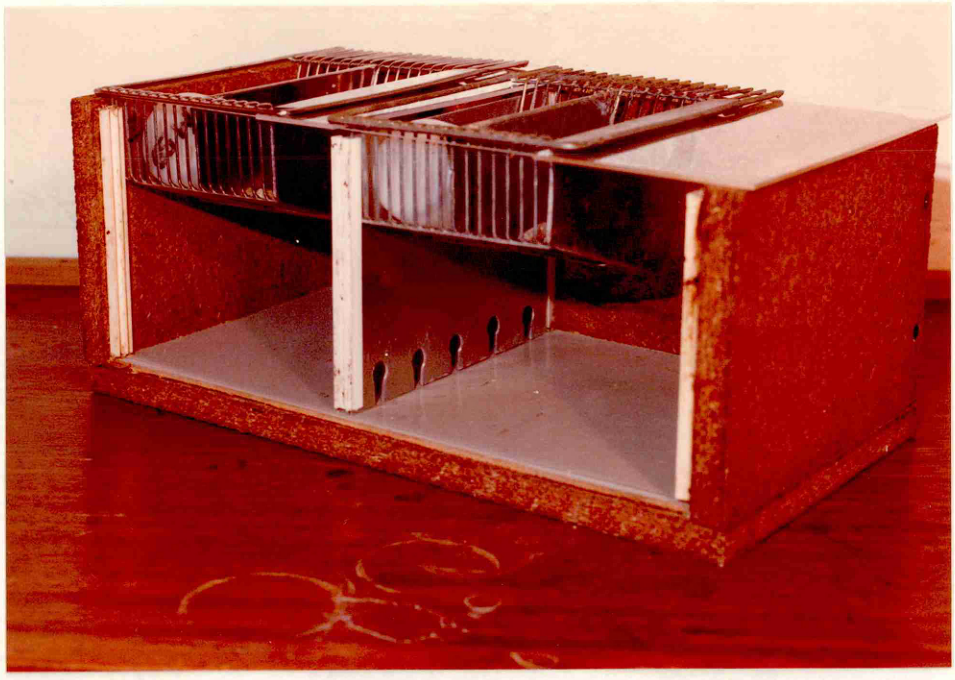


Fig. 6.e The Split Cage (empty)

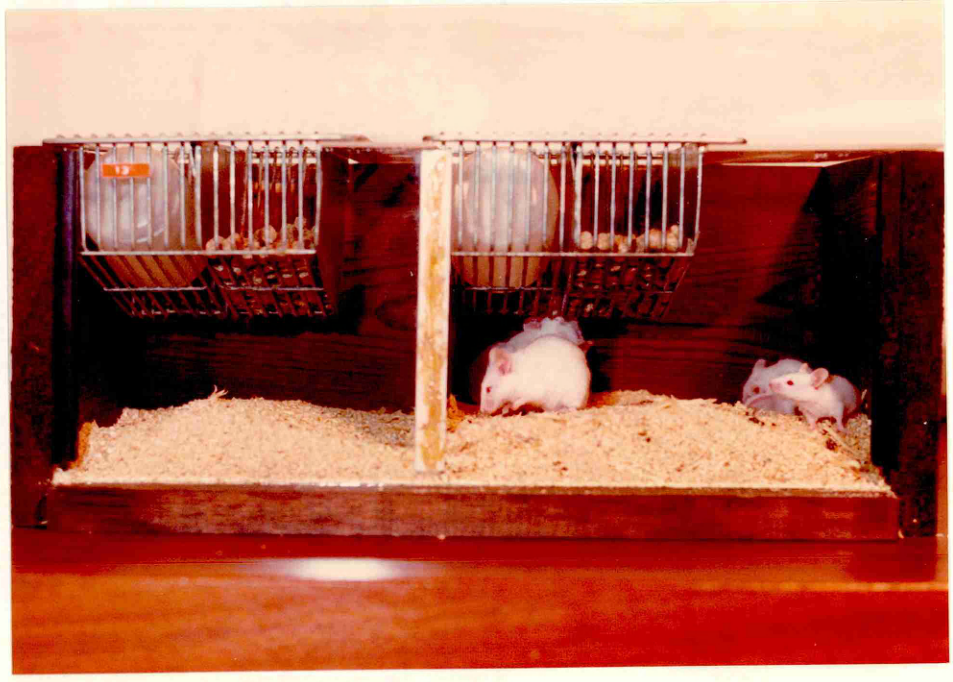


Fig. 6.f The Split Cage (with subjects)

Fig. 6.g The Split Cage, showing the right hand compartment

Fig. 6.h The Split Cage, showing the left hand compartment

Fig. 6.i The Split Cage, with pup moving through pup filter hole

Fig. 6.j The Split Cage, with pup emerging from pup filter hole

to those described in Chapter 2.

The cage proved totally adequate for the experiment, and the design is suitable for future research. It has, however, a number of characteristics which are worth noting.

Only when the pups are mobile are they able to gain access to the father compartment. This means that for the first few days of life the pups will be subjected only to the influence of the parent(s) in the mother compartment. According to Williams & Scott (1953), mouse pups begin to leave the nest area around day 8.

The Split Cage allows for behavioural recordings to be made with the minimum of disturbance to the subjects. With the Split Litter experiment, the pups were handled twice daily in order to transfer them to their appropriate cages. By contrast, once litter sizes and parental composition were determined soon after the litter was born, no further handling of the animals was required with the Split Cage experiment.

The cage design enables recordings to be made of the behaviour of both the parents and the offspring. The large perspex front to the cage provides exceptionally good visual access to the cage contents.

Experimental Procedure. A total of 12 litters and their parents were used, which provided four replicates for each experimental condition. In order to keep maternal experience constant in this experiment, all mothers used were multiparous. All of the pairs of parents used had been together since their original mating. As soon as the female of a breeding pair became noticeably pregnant, both the

mother and the father were placed into the mother compartment of one of the cages. As soon as the litter was born, the father's position was determined, according to which of the three treatment conditions was applicable. The father was either retained in the right hand compartment with the mother and litter (FRI); moved through to the left hand compartment (FLE); or removed completely so that there was no father influence at all (NOF). In the conditions where the father was retained, a spot of dark blue fur marker was placed on the back of his neck in order to aid identification during the observation sessions. Where necessary, the litter size was reduced to seven on day 2, in order to keep the litter size constant throughout the experiment and identical to the litter size used in the main experiment. The removal of pups on day 2 does not appear to affect the normal parental behaviour (Chapter 2).

Day 10 marked the onset of the recording period in both the main experiment and the Split Litter experiment. The decision to delay until day 10 (Chapter 2) was made because of the difficulties encountered in observing the behaviour of a single focal subject pup at an earlier age. At a very young age, subject pups are obscured for a large proportion of time by their parents and littermates. Changed circumstances in the Split Cage experiment, however, prompted an earlier beginning to the recording period. The Split Cage experiment represents the first deliberate attempt in this study to quantitatively measure the parents' behaviour and no specific problems are encountered in measuring parental behaviour at an early pup age. Further, the focus of attention during the observation sessions was not a single focal pup, but instead, the whole litter. By the time this experiment was ready to be conducted, I had become much more familiar with the behaviour of young pups and could determine more

effectively exactly what activity they were engaged in during any single scan. Characteristic postures are adopted for particular activities which can quickly be identified. Drewett et al. (1974) referred to the way that suckling can quickly be recognized by the posture of the pup, which for most of the suckling episode lies very still. I decided, in view of these changed circumstances, to record both the parents' and the offspring's behaviour from day 3. Recordings were then continued on a daily basis until day 30, similar to the Split Litter experiment.

Daily Recordings. All recordings were carried out under red light, towards the end of the night phase of the day/night cycle; as described in Chapter 2. An instantaneous sampling technique was used which was similar to that adopted for the main experiment, but where a 10 second time interval, as opposed to 5 seconds, separated successive audible signals. The behavioural state of the animals at the time of the signal was recorded. During each recording session the total time required for 100 instantaneous sampling scans was 16 minutes 40 seconds. The following information was recorded from each scan.

1. Whether the parents were present in the nest. Both parents hollowed out a depression in the sawdust, but at least one pup had to be present for this area to constitute a nest (score obtained for each parent). These scores also enabled a comparison to be made in the FRI condition between the proportions of time spent by only the mother in the nest, only the father in the nest, and both parents in the nest.

2. Whether the parents were stimulating the pup(s). This included such caretaking behaviour as licking, sniffing, grooming, retrieving, stepping on pups etc. (score obtained for each parent).

3. Whether parents were involved in nest construction. This included the formation of a depression in the sawdust, the collection of woodshavings and the manipulation of nesting material with the snout and forepaws (score obtained for each parent).

4. Whether the mother or the father was displaying an activity which was not directly associated with caretaking behaviour (called non-caretaking activities). This included locomotion, exploratory behaviour, self-grooming, feeding, drinking etc. (score obtained for each parent).

5. The number of pups present in the nest (score obtained for both compartments).

6. Proximity to parent. The number of pups in contact with, or within 1cm, of a parent (score obtained for both compartments).

7. The number of pups suckling, including active nipple-seeking (score obtained only in mother compartment).

8. The number of pups eating solid food (score obtained for both compartments).

9. The number of pups exhibiting locomotor behaviour (score obtained for both compartments).

10. The number of pups exhibiting exploratory behaviour; involving sniffing, head-lifting and rearing (score obtained for both compartments).

11. The number of pups exhibiting self-grooming; including scratching and licking (score obtained for both compartments).

12. The number of pups in the left compartment.

The behaviour observed during each scan was recorded on a check sheet during the interval between scans. At the end of the recording sessions the scores in each of the behaviour categories were totalled. For the parent activities, a maximum score of 100 could be obtained, but for the offspring activities, since there were seven pups in each litter, a maximum score of 700 could be obtained. These proportions of 700 were converted to percentages so that the mean percentages of the pup scores could be shown on the graphs of these data (Figs. 6.27 to 6.34). In common with the procedure adopted for the previous experiments, the mean of each two successive days' scores was calculated. This provided 14 scores for each activity from the total 28 day recording period. Fewer categories of pup behaviour have been recorded than in the previous experiments in order to provide sufficient time for the simultaneous recording of parent and offspring behaviour. The three categories of exploratory behaviour have been contracted into one category. Because only very low drinking scores had been obtained in previous work, this category was eliminated.

Day 30 Measurements. On day 30, open field tests were administered and body weights were measured for all the offspring. The type of measures taken were identical to those in the Split Litter experiment.

Fig. 6.k summarizes the design of the Split Cage experiment.

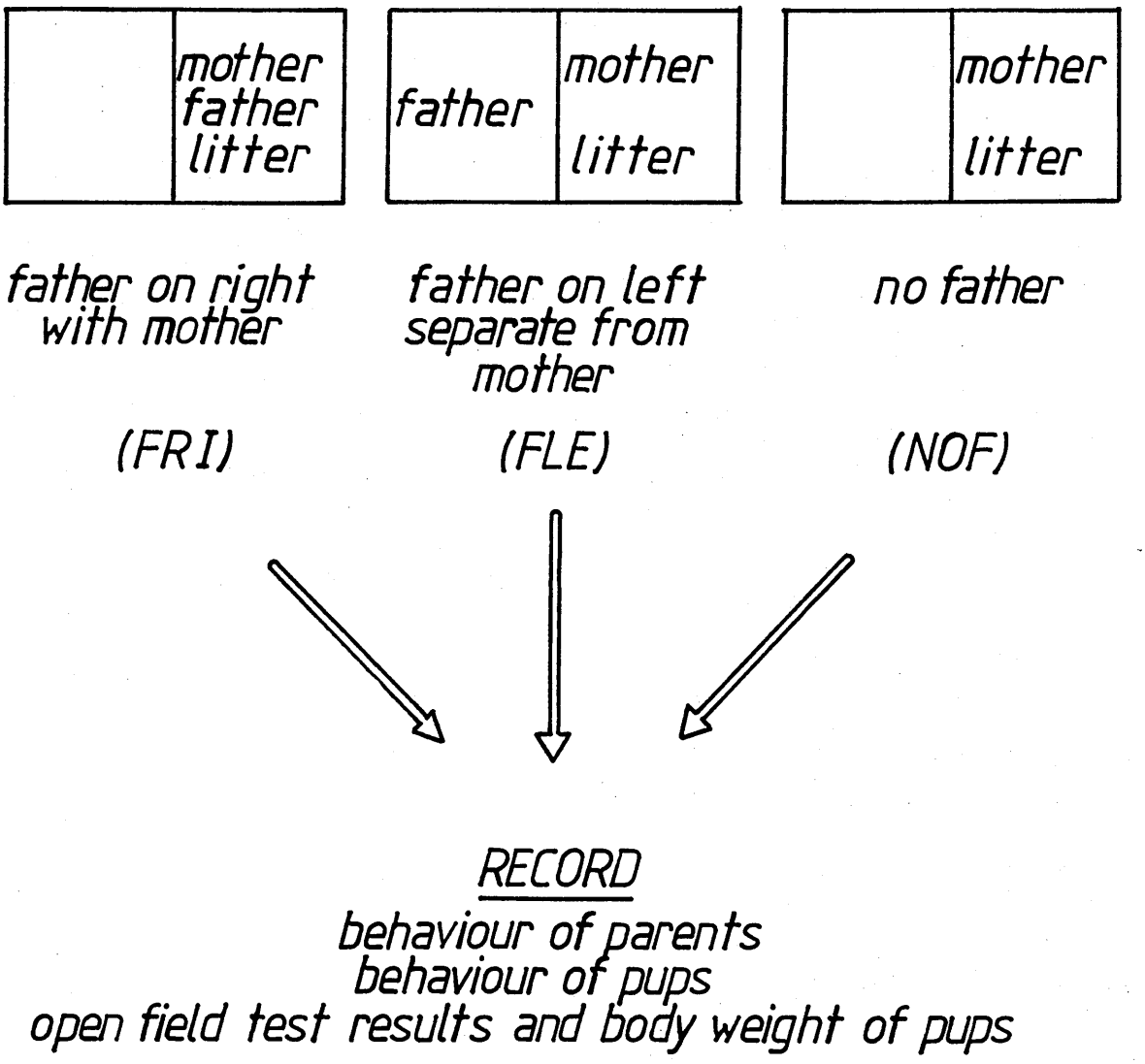


Fig. 6.k Split Cage experiment design.

Data Treatment. The data were treated in a similar fashion to that adopted for the Split Litter experiment. The data from the daily observations were transformed (ARCSIN) before analyses of variance were conducted. Age was again treated as a covariate, which resulted in a one-way ANOVA with 'position of father' as the independent variable. Pearson product moment correlation analyses were administered between individual pup scores for behaviours under each separate experimental treatment, and pup age. The correlation analyses together with the covariate analyses were used to investigate age trends. A total of 17 analyses were conducted on the daily observations data. Eight analyses of variance were also carried out on the untransformed litter means of the day 30 data. Scheffé tests were administered whenever a significant difference was found with the ANOVA.

Results

Table 6.B provides a summary of all the age analyses. It shows significant age effects for the measures, as revealed by the analysis of covariance. These are general age effects, across all three experimental treatments, for each behavioural activity. In addition to this, Table 6.B shows, with the correlation analyses, how the age effects are distributed between the different treatments. Tables 6.18 to 6.42 summarize the results of the analyses of variance, where the same symbols and conventions are used as in the Split Litter tables. (Tables 6.18 to 6.34 are based on transformed data. In these tables, backtransformed means, expressed as percentages, are provided in parentheses.) Again it should be noted that Fig. numbers correspond to Table numbers. For example, Table 6.18 and Fig. 6.18 are based on the same data. Figs. 6.18 to 6.34 show the mean

Table 6.B Age Analyses For Split Cage Treatment Effects

Values for F, df, and P are shown for the Covariate analyses

Values for r (correlation coefficient) and P (two-tailed) are shown for the Pearson Correlation analyses

MEASURE & Covariate values		TREATMENTS & Correlation values FRI	FLE	NOF
MOTHER IN NEST	r	-0.216	0.148	0.392
F=2.181 df=1,164 NS	P	NS	NS	<0.01
MOTHER, STIMULATING PUP	r	-0.219	-0.247	-0.389
F=18.776 df=1,164 P<0.001	P	NS	NS	<0.01
MOTHER, NEST CONSTRUCTION	r	-0.485	-0.318	-0.264
F=25.175 df=1,164 P<0.001	P	<0.001	<0.05	=0.05
MOTHER, NON-CARETAKING	r	-0.022	-0.077	-0.239
F=2.122 df=1,164 NS	P	NS	NS	NS
FATHER IN NEST	r	-0.167	0.056	
F=0.917 df=1,109 NS	P	NS	NS	
FATHER, STIMULATING PUP	r	-0.298	0.225	
F=2.326 df=1,109 NS	P	<0.05	NS	
FATHER, NEST CONSTRUCTION	r	-0.157	0.235	
F=1.664 df=1,109 NS	P	NS	NS	
FATHER, NON-CARETAKING	r	0.230	-0.067	
F=0.586 df=1,109 NS	P	NS	NS	
PARENTS IN NEST	r	-0.091	0.112	-0.177
F=0.847 df=1,164 NS	P	NS	NS	NS
PUPS IN NEST	r	-0.615	-0.554	-0.396
F=91.125 df=1,164 P<0.001	P	<0.001	<0.001	<0.01
PUPS WITH PARENT	r	-0.304	0.255	0.500
F=6.165 df=1,164 P<0.05	P	<0.05	NS	<0.001
PUPS SUCKLING	r	-0.198	0.036	0.354
F=0.180 df=1,164 NS	P	NS	NS	<0.01
PUPS EATING SOLID FOOD	r	0.563	0.525	0.453
F=92.164 df=1,164 P<0.001	P	<0.001	<0.001	<0.001
PUPS, LOCOMOTOR BEHAVIOUR	r	0.422	-0.280	-0.629
F=1.429 df=1,164 NS	P	<0.01	<0.05	<0.001
PUPS, EXPLORATORY BEHAVIOUR	r	0.626	0.543	0.665
F=182.084 df=1,164 P<0.001	P	<0.001	<0.001	<0.001
PUPS, GROOMING	r	0.683	0.648	0.576
F=215.709 df=1,164 P<0.001	P	<0.001	<0.001	<0.001
PUPS IN LEFT COMPARTMENT	r	0.401	0.347	0.184
F=17.952 df=1,164 P<0.001	P	<0.01	<0.01	NS

FRI: father in the right compartment
FLE: father in the left compartment
NOF: no father present

percentage of instantaneous scans of the parents and of the pups involved in the activities which were recorded on a daily basis. These values are plotted against pup age. Each number on the 'pup age' axis refers to the first day of the pair of days from which each result had been obtained. For example, '19' refers to the overall score obtained by averaging the two scores from days 19 and 20. Figs. 6.35 to 6.42 are histograms based on the day 30 data. Of the 25 analyses carried out, 16 involved significant differences. These 16 significant results are summarized in Table 6.c.

Where symbols FRI, FLE and NOF are used, they refer respectively to 'father in the right compartment', 'father in the left compartment' and 'no father present'. The symbols M and F refer respectively to mother and father, and are used in the analysis comparing the time spent by the two FRI parents in the nest (Table 6.26 and Fig. 6.26). All graphs are based on untransformed data.

The tables and graphs are preceded by a summary of all of the results, and are followed by a summary table (Table 6.c) showing the significant differences (ANOVA) between the treatment groups. These differences are then considered in more detail in the discussion section which follows.

Summary of Results. Of the 25 analyses of variance carried out, 16 involved significant differences. (The numbers accompanying the behaviour headings, below, match the Table and Fig. numbers.)

18. Mother in Nest. The FRI mean was significantly greater than both the FLE mean and the NOF mean, and the FLE mean was significantly greater than the NOF mean (Table 6.18). The analysis of covariance revealed that no significant age effect was apparent

for this activity and the correlation analyses showed that only in the case of the NOF scores was there a significant positive correlation between the activity scores and age (Table 6.B). Fig. 6.18 also shows that the change with age of the NOF scores is different to that of the other two groups. The unexpected upward trend in the NOF scores is a result of the NOF mothers spending very little time in the nest with their offspring when the pups were very young (especially days 3 to 8), but spending increasing amounts of time in the nest as the pups' age increased. This may suggest an initial aversion to the pups in the nest, but an aversion nevertheless that declined with continued exposure to the offspring or with increased pup age. The NOF mothers' propensity to enter the nest may also have been inversely related to the number of pups in the nest. This would mean that as the pups began to spend more time out of the nest, the mother spent more time in the nest. The question of why it was just the NOF mothers which displayed an early aversion to pups is addressed later.

19. Mother, Stimulating Pup. There was no significant difference between the scores obtained from the three treatment groups (Table 6.19). The analysis of covariance revealed a significant age effect, but only in the NOF correlation analysis was a significant negative correlation apparent (Table 6.B). It would be anticipated that the attention given to offspring by the mother would decrease with pup age, but Fig. 6.19 shows that the downward trend, apparent in the NOF scores, is obscured by high variability in the FRI and FLE scores.

20. Mother, Nest Construction. The ANOVA indicated that there was a significant difference between the treatment scores, although the Scheffé test result did not show this difference (Table 6.20). A comparison of the mean scores showed that the FRI and FLE means had the same value and that this was larger than the NOF mean. The

analysis of covariance revealed a significant age effect and the correlation analyses showed a significant negative correlation between this activity and age for all treatment conditions (Table 6.B). The NOF correlation was only marginally significant. It would be expected that maternal nest construction would decrease with pup age as the functional need for a nest decreased. Fig. 6.20 shows that nest construction was not displayed by NOF mothers past day 7, and not displayed by FLE mothers past day 17. FRI mothers continued to display this activity to the end of the recording period (days 29/30). The presence of the father appeared to stimulate the mother to continue nest-building for a longer period of time. This point is discussed in more detail later.

21. Mother, Non-Caretaking. Both the FLE and NOF means were significantly greater than the FRI mean (Table 6.21). The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.B).

22. Father in Nest. The FRI mean was significantly greater than the FLE mean (Table 6.22). The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.B).

23. Father, Stimulating Pup. Again the FRI mean was significantly greater than the FLE mean (Table 6.23). The analysis of covariance showed that no significant age effect was apparent for this activity and the correlation analyses showed that only in the case of the FRI scores was there a significant negative correlation between the activity scores and age (Table 6.B). Fig. 6.23 shows that a large difference between the FRI and FLE scores was apparent at an early pup age. The FRI fathers had access to their offspring throughout

the development period whereas the FLE fathers only had access to the offspring once the pups were sufficiently developed to move through to the father compartment. Differing accessibility to the offspring for the two groups of fathers has not unexpectedly resulted in dissimilar patterns of paternal pup stimulation with age (Fig. 6.23).

24. Father, Nest Construction. There was no significant difference between the scores obtained from the two treatment groups (Table 6.24). The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.B).

25. Father, Non-Caretaking Activity. The FLE mean was significantly greater than the FRI mean (Table 6.25). This result is related to the fact that the pups were less accessible to the FLE fathers than to the FRI fathers. The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.B).

26. Parents in Nest. This is where a comparison was made, in the FRI condition, between the proportion of time spent by only the mother in the nest (M), only the father in the nest (F), and both parents in the nest (M & F). Table 6.26 shows that the 'M & F' mean was significantly greater than the 'M' mean and significantly greater than the 'F' mean. The analysis of covariance and the correlation analyses showed that no significant trend with age was apparent for this activity (Table 6.B).

27. Pups in Nest. The NOF mean was significantly greater than the FRI mean (Table 6.27). The analysis of covariance revealed a significant age effect and the correlation analyses showed a significant negative correlation between this activity and age for

all treatment conditions (Table 6.B). It would be expected that a decrease in time spent by the pups in the nest would correspond with an increase in pup age. Fig. 6.27 indicates that it was the FRI pups which were observed leaving the nest first (days 15/16). The NOF pups were not observed leaving the nest until days 23/24.

28. Pups with Parent. The FRI mean was significantly greater than both the FLE and the NOF means and the FLE mean was significantly greater than the NOF mean (Table 6.28). The analysis of covariance revealed a significant age effect, and the correlation analyses showed differing age trends across the three treatment conditions (Table 6.B). A significant negative correlation with age was found for the FRI pups, a significant positive correlation for the NOF pups and no significant correlation at all for the FLE pups. Differing treatment circumstances for the three groups would have been mainly responsible for these correlation differences. From the beginning of the recording period the FRI pups had access to both parents. Fig. 6.28 shows how this has resulted in high scores, at the beginning of the recording period, which have gradually declined throughout the period. The NOF trend, where the only parent is the mother, shows the opposite and, not surprisingly, is similar to the trend obtained from the 'mother in nest' scores (see section 18, above).

29. Pups, Suckling. The FRI mean was significantly greater than the NOF mean (Table 6.29). No age effect was apparent with the analysis of covariance for this activity, and the correlation analyses showed that only in the case of the NOF scores was there a significant positive correlation between the activity scores and age (Table 6.B). This NOF trend (Fig. 6.29) is the opposite to that which would be anticipated for developing pups, but nevertheless is consistent with

the 'mother in nest' and 'pups with parent' age trends which have been discussed above.

30. Pups Eating Solid Food. There was a significant difference between the treatment scores according to the ANOVA, although the Scheffé test did not show this difference (Table 6.30). A comparison of the mean scores showed that the FRI pups had the largest mean and the NOF pups had the smallest. The analysis of covariance revealed a significant age effect and the correlation analyses showed a significant positive correlation between this activity and age for all treatment conditions (Table 6.B). Such a result is as would be anticipated for this activity. Fig. 6.30 shows that it was the FRI pups which began eating solid food first (days 13/14). NOF pups did not begin eating solid food until days 23/24.

31. Pups, Locomotor Behaviour. There was no significant difference between the scores obtained from the three treatment groups (Table 6.31). The analysis of covariance showed that no significant age effect was apparent for this activity (Table 6.B). The correlation analyses revealed a significant positive correlation for the FRI scores with age, whereas significant negative correlations with age were found for the FLE and NOF scores. It must be concluded that the difference in trends is directly attributable to the father's position in the cage. It should be noticed that the direction of these correlations is the opposite to those obtained from the 'pups with parent' data (Table 6.B and Fig. 6.28). It is possible, therefore, that locomotion varied inversely with the amount of pup contact with parents.

32. Pups, Exploratory Behaviour. The FRI mean was significantly greater than the FLE mean (Table 6.32). The analysis of covariance revealed a significant age effect and the correlation analyses

showed a significant positive correlation between this activity and age for all treatment conditions (Table 6.B).

33. Pups, Grooming. There was no significant difference between the scores obtained from the three treatment groups (Table 6.33). The analysis of covariance revealed a significant age effect and the correlation analyses showed a significant positive correlation between this activity and age for all treatment conditions (Table 6.B).

34. Pups in Left Compartment. The FLE mean was significantly greater than the NOF mean (Table 6.34). The analysis of covariance revealed a significant age effect and the correlation analyses showed a significant positive correlation between this activity and age in the FRI and FLE groups (Table 6.B). There was no significant correlation with age for the NOF group and this is not surprising given the very low scores for this group (Fig. 6.34).

35. Open Field, Peripheral Compartment Ambulation. There was no significant difference between the scores obtained from the three treatment groups.

36. Open Field, Inner Compartment Ambulation. The FRI mean was significantly greater than the FLE mean (Table 6.36).

37. Open Field, Rearing. There was no significant difference between the scores obtained from the three treatment groups.

38. Open Field, Latency to Defaecation. The FRI mean was significantly greater than both the FLE and the NOF means (Table 6.38).

39. Open Field, Defaecation. There was no significant difference between the scores obtained from the three treatment groups.

40. Open Field, Latency to Urination. Both the FLE and FRI means were significantly greater than the NOF mean (Table 6.40).

There was no significant difference between the scores obtained from the three treatment groups in:

41. Open Field Urination and

42. Body Weight.

Table 6.18 Analysis of Variance of 'Mother in Nest' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	5.086	18.075	< 0.001
RESIDUAL	164	0.281		

Position of Father:	NOF	FLE	FRI
Means:	0.406	0.668	1.007
	(15.06)	(38.37)	(71.44)

Table 6.19 Analysis of Variance of 'Mother, Stimulating Pup' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.052	2.703	NS
RESIDUAL	164	0.019		

Table 6.20 Analysis of Variance of 'Mother, Nest Construction' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.010	3.671	< 0.05
RESIDUAL	164	0.003		

Position of Father:	NOF	FLE	FRI
Means:	0.004	0.028	0.028
	(0.002)	(0.08)	(0.08)

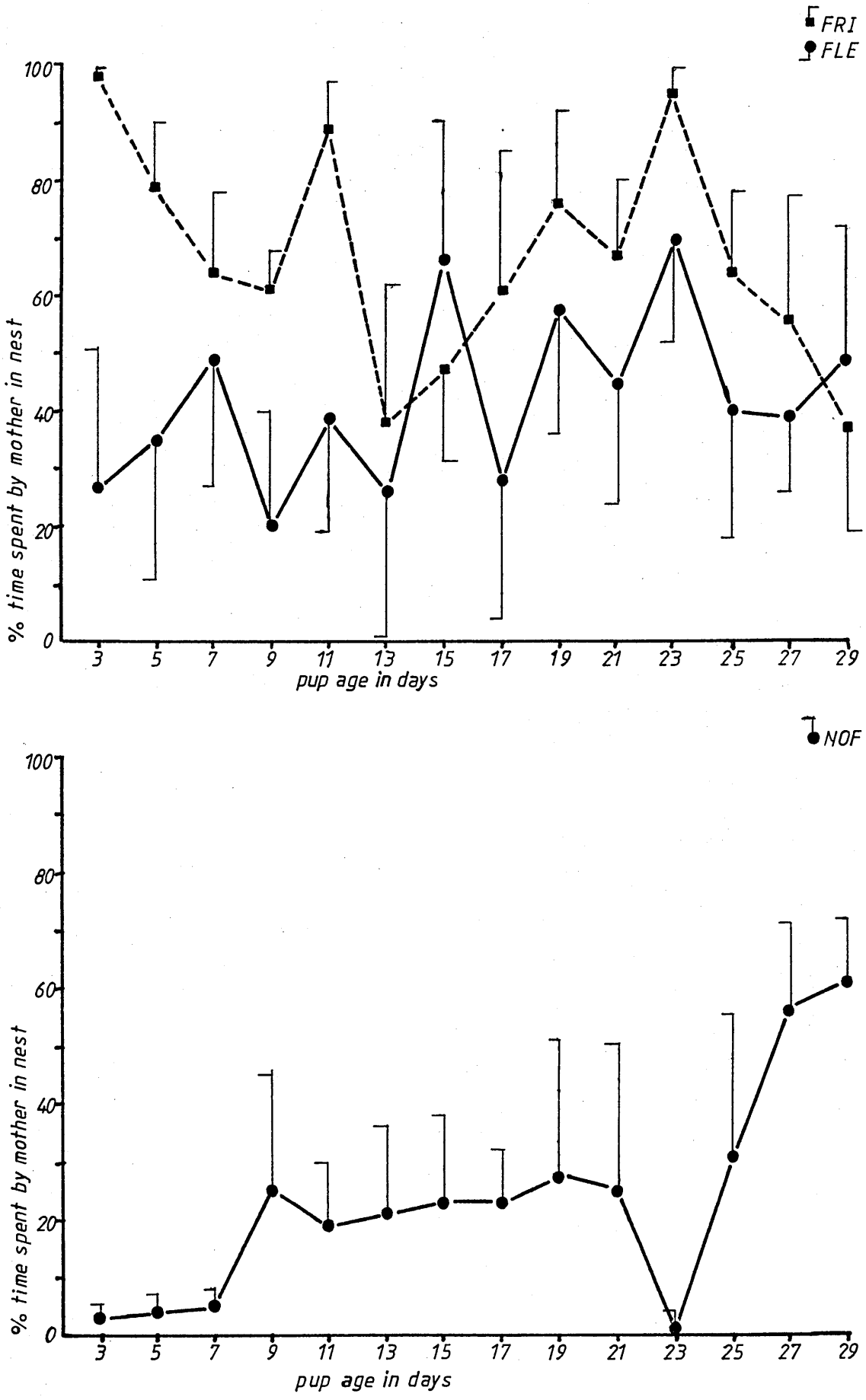


Fig. 6.18 *Mean percentage of instantaneous scans of mother in nest. Standard errors are indicated.*

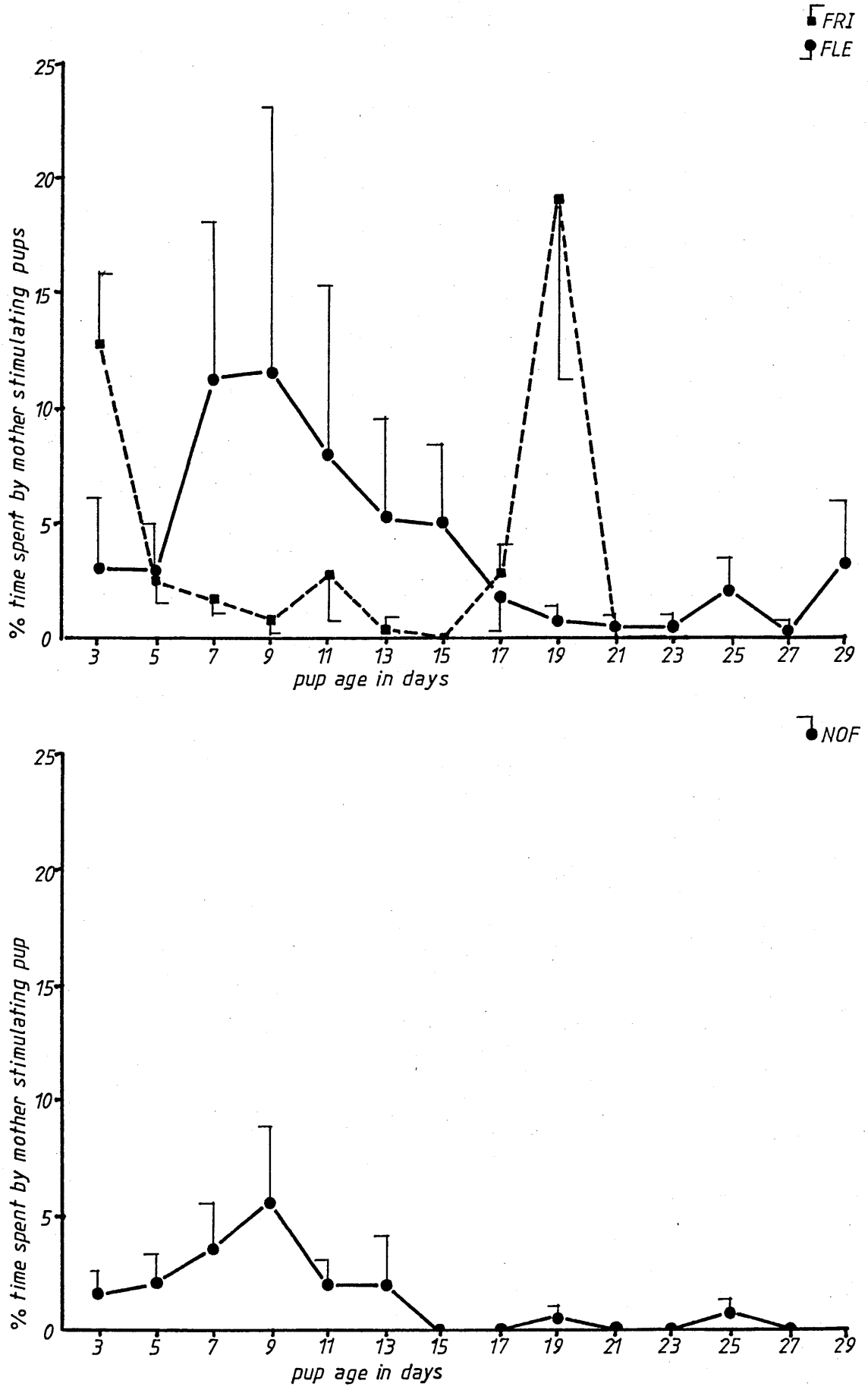


Fig. 6.19 *Mean percentage of instantaneous scans of mother stimulating pup. Standard errors are indicated.*

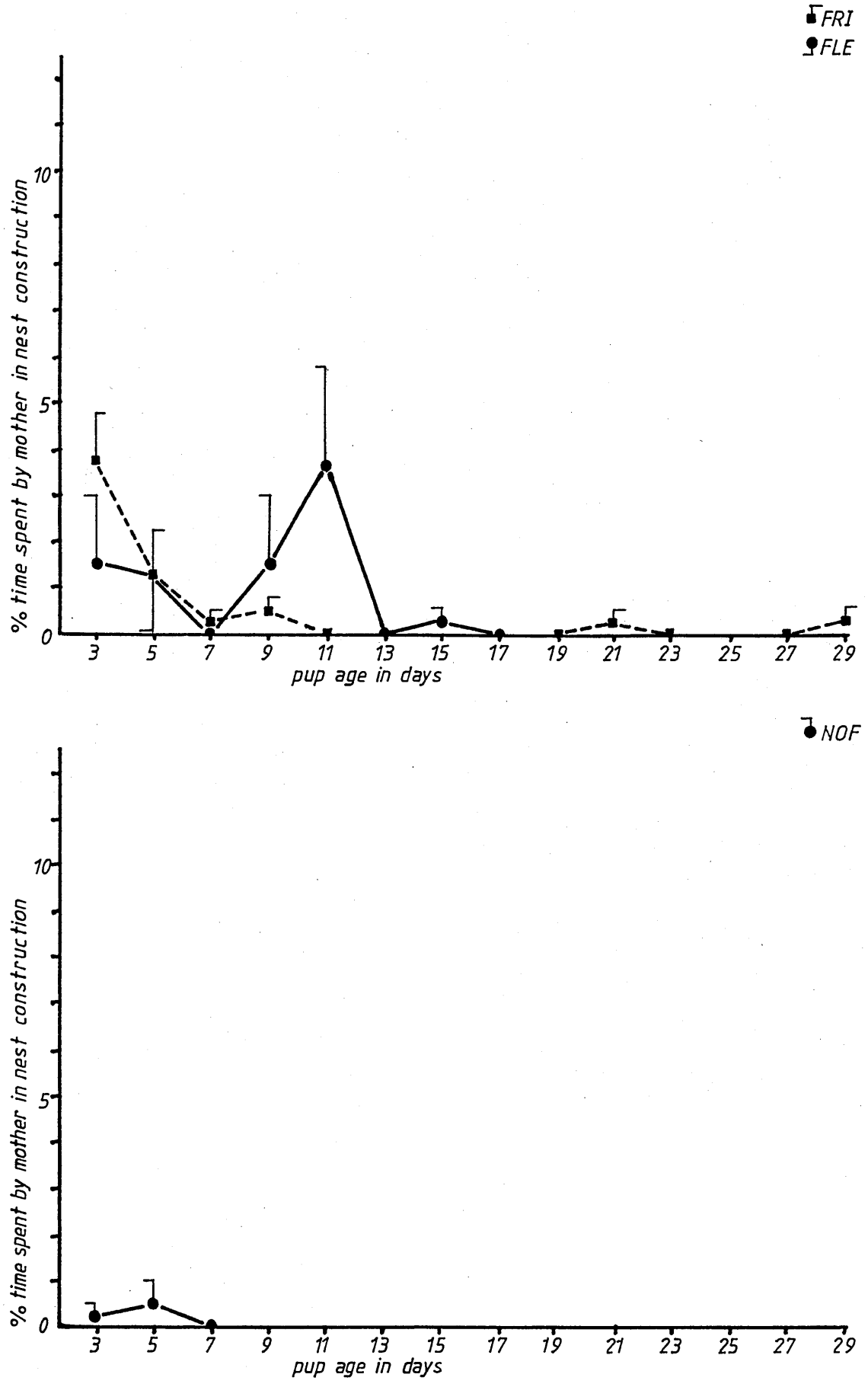


Fig. 6.20 *Mean percentage of instantaneous scans of mother involved in nest construction. Standard errors are indicated.*

Table 6.21 Analysis of Variance of 'Mother, Non-Caretaking Activity' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	3.955	20.467	< 0.001
RESIDUAL	164	0.193		

Position of Father:	FRI	FLE	NOF
Means:	0.732	1.066	1.258
	(44.67)	(76.61)	(90.53)

Table 6.22 Analysis of Variance of 'Father in Nest' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	1	24.180	160.674	< 0.001
RESIDUAL	109	0.150		

Position of Father:	FLE	FRI
Means:	0.070	0.999
	(0.49)	(70.72)

Table 6.23 Analysis of Variance of 'Father, Stimulating Pup' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	1	0.409	18.017	< 0.001
RESIDUAL	109	0.023		

Position of Father:	FLE	FRI
Means:	0.031	0.152
	(0.10)	(2.29)

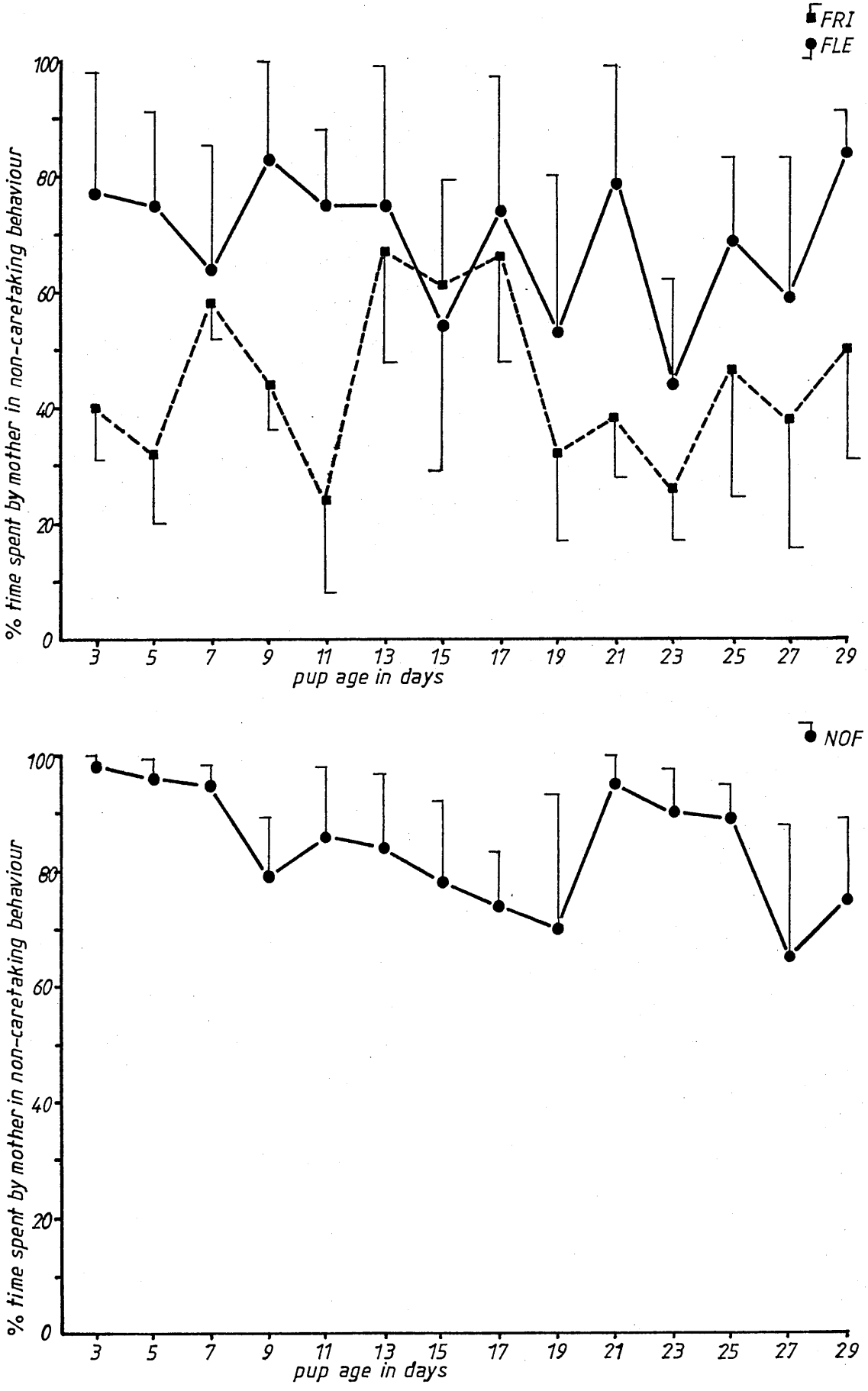


Fig. 6.21 *Mean percentage of instantaneous scans of mother exhibiting non-caretaking behaviour. Standard errors are indicated.*

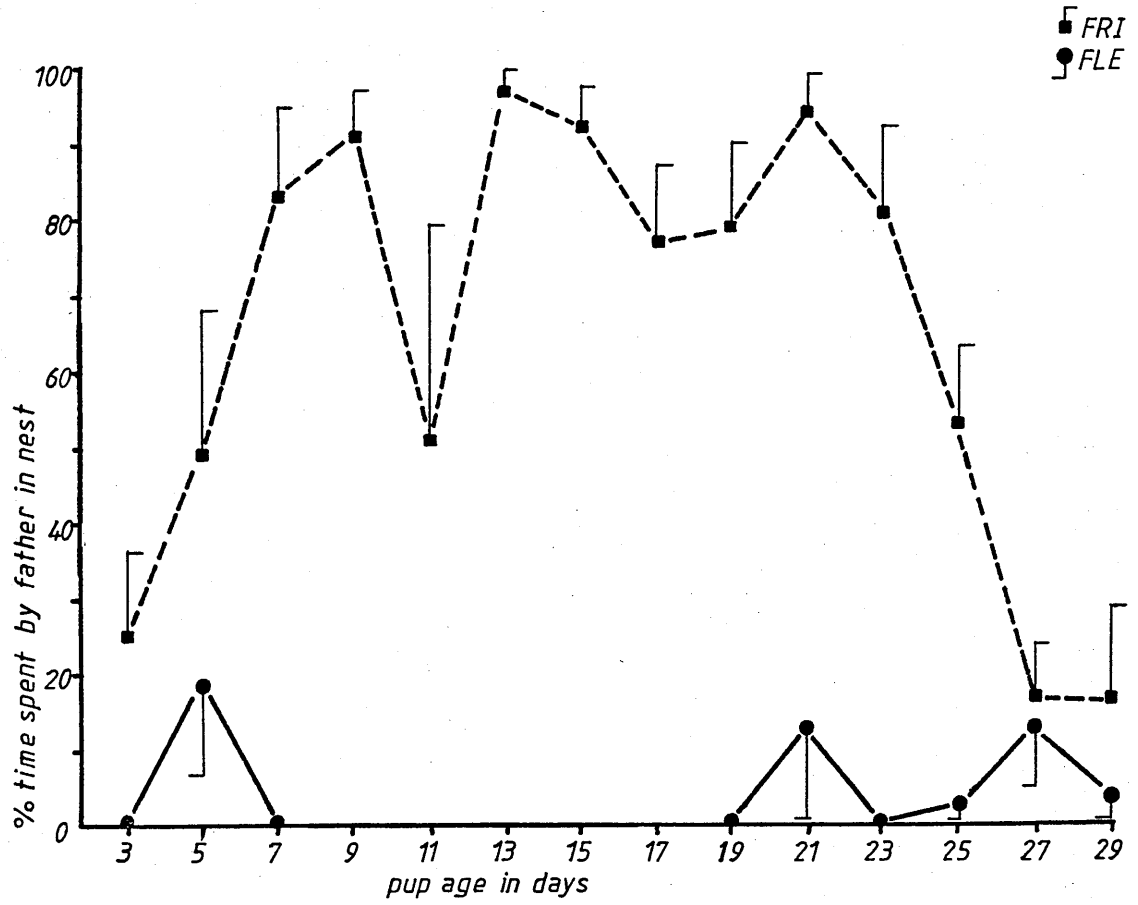


Fig 6.22 *Mean percentage of instantaneous scans of father in nest. Standard errors are indicated.*

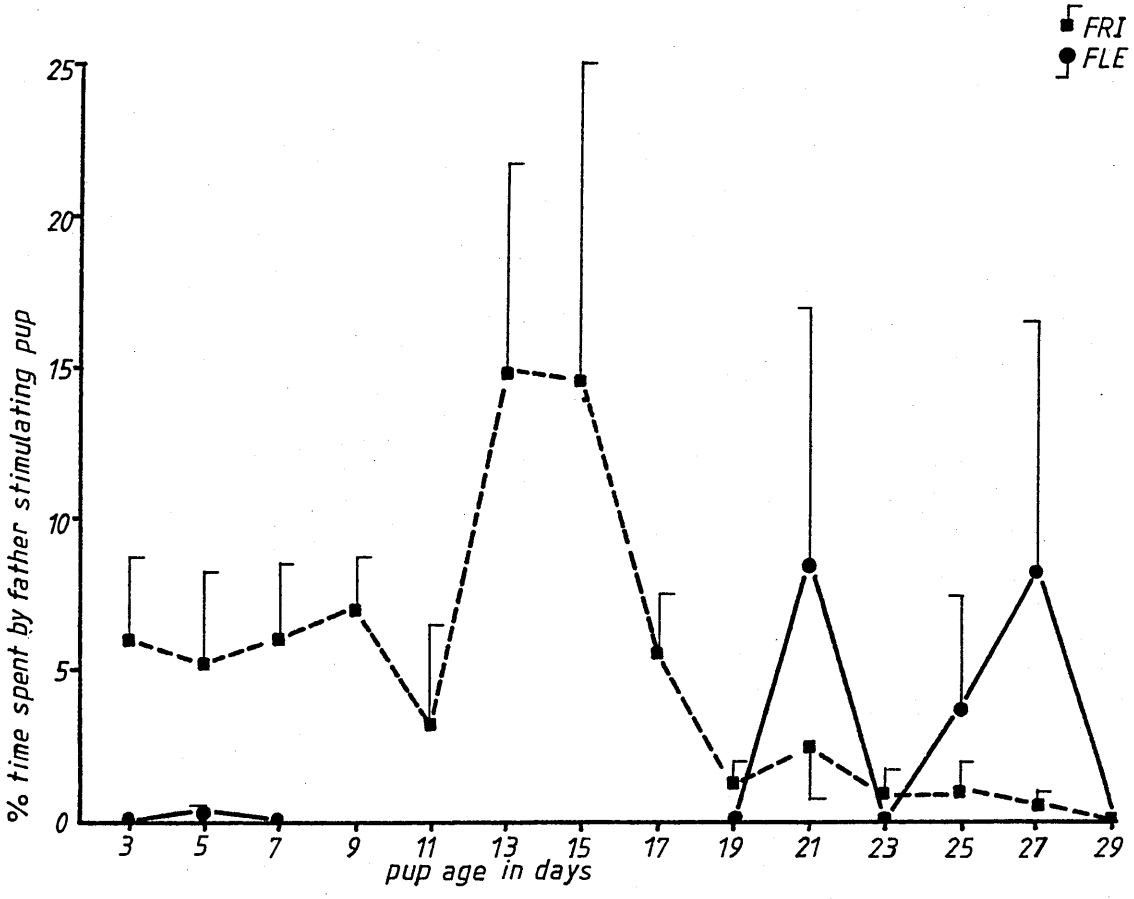


Fig.6.23 Mean percentage of instantaneous scans of father stimulating pup. Standard errors are indicated.

Table 6.24 Analysis of Variance of 'Father, Nest Construction' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	1	0.022	3.087	NS
RESIDUAL	109	0.007		

Table 6.25 Analysis of Variance of 'Father, Non-Caretaking Activity' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	1	11.915	73.718	<0.001
RESIDUAL	109	0.162		

Position of Father:	FRI	FLE
Means:	0.709	1.362
	(42.39)	(95.70)

Table 6.26 Analysis of Variance of 'Parents in Nest' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	3.080	17.782	<0.001
RESIDUAL	164	0.173		

Parents in Nest:	M Only	F Only	M & F
Means:	0.311	0.336	0.729
	(9.36)	(10.78)	(44.37)

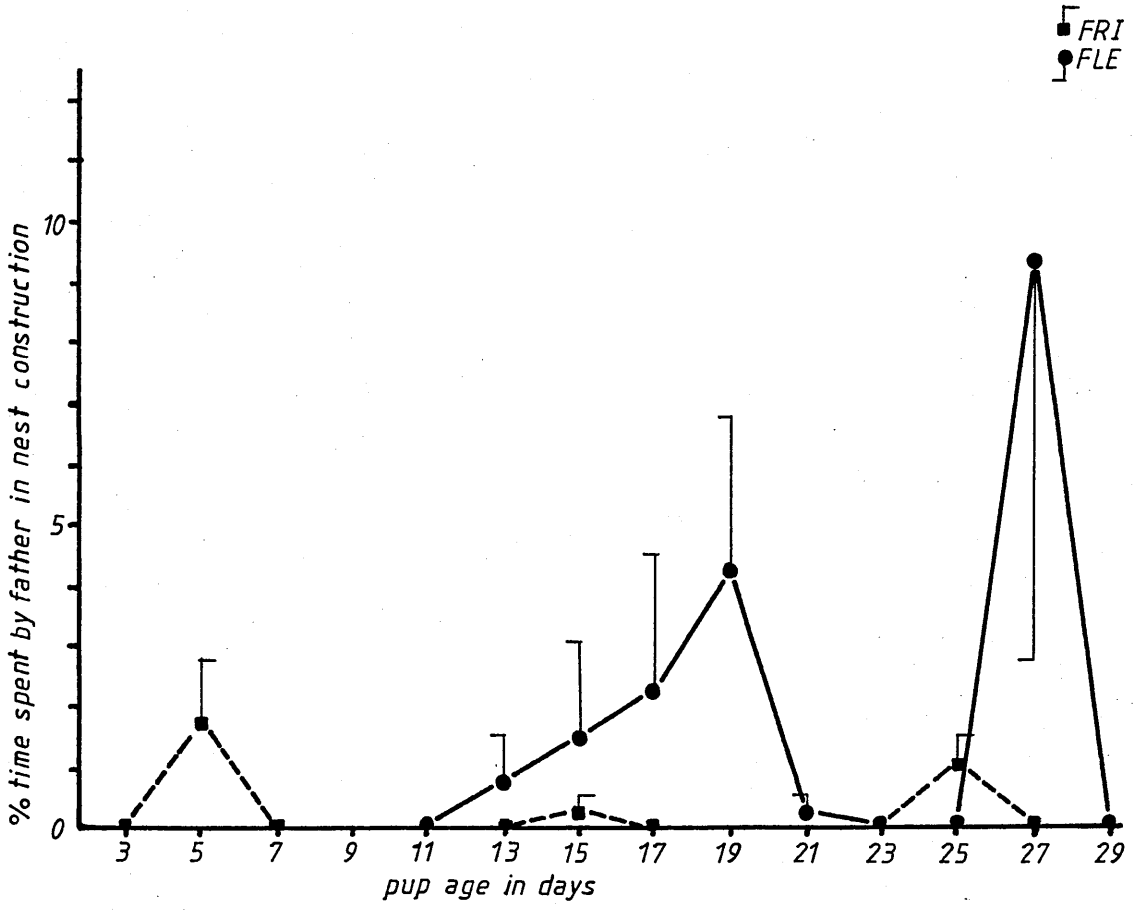


Fig. 6.24 *Mean percentage of instantaneous scans of father involved in nest construction. Standard errors are indicated.*

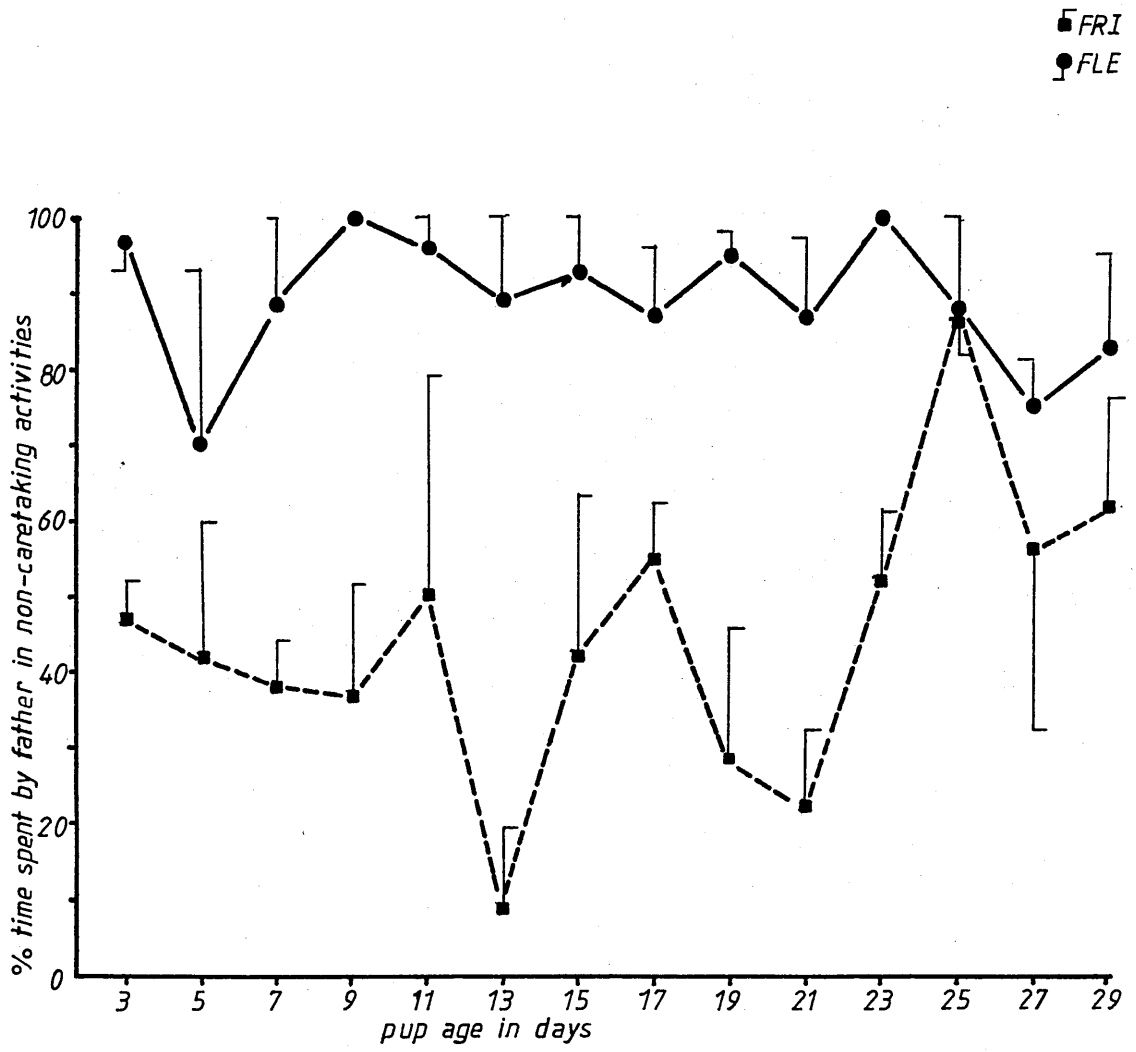


Fig. 6.25 Mean percentage of instantaneous scans of father exhibiting non-caretaking activities. Standard errors are indicated.

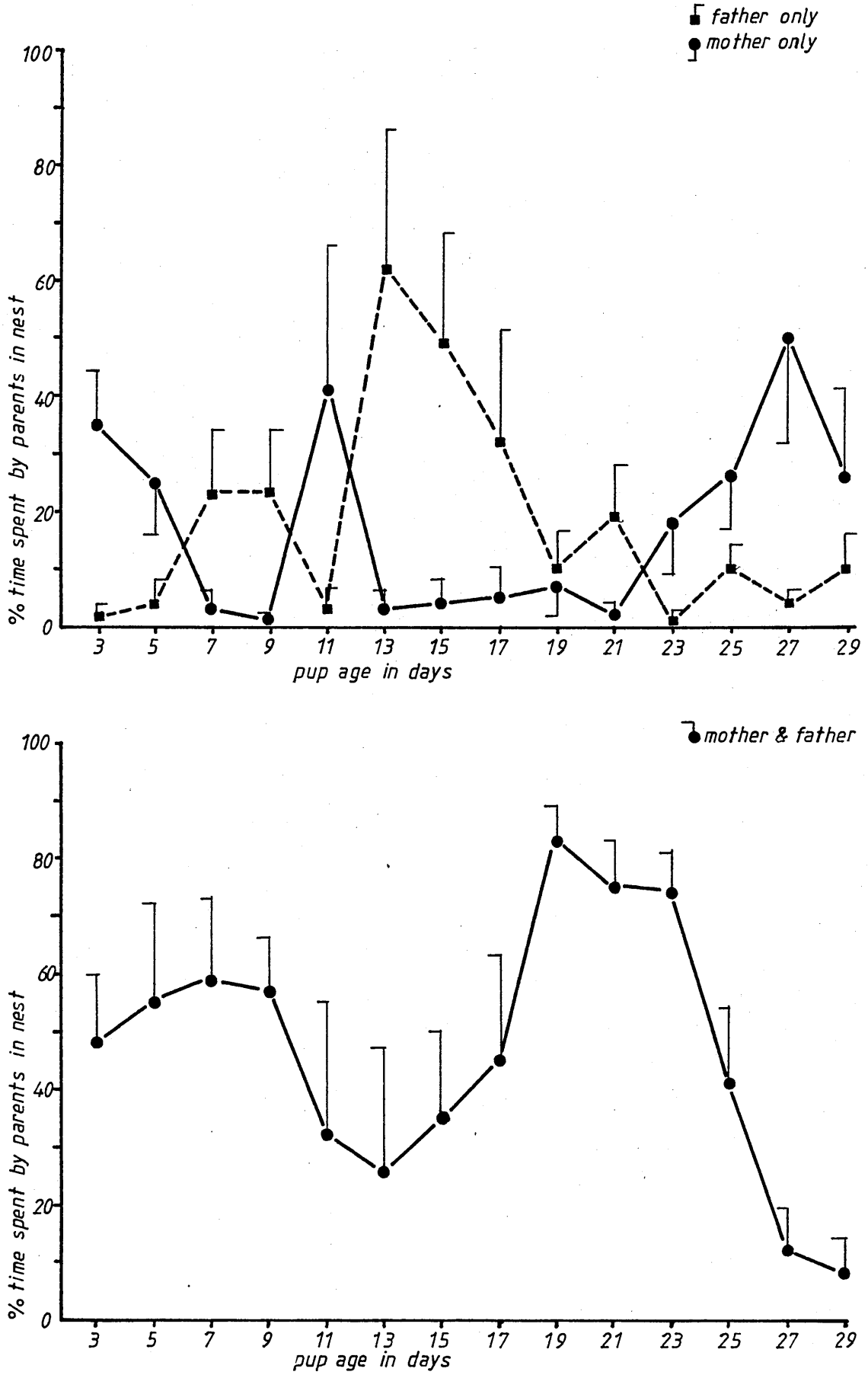


Fig. 6.26 *Mean percentage of instantaneous scans of parents in nest. Standard errors are indicated.*

Table 6.27 Analysis of Variance of 'Pups in Nest' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.332	3.974	<0.05
RESIDUAL	164	0.084		

Position of Father:	FRI	FLE	NOF
Means:	1.323	1.403	1.477
	(93.98)	(97.21)	(99.12)

Table 6.28 Analysis of Variance of 'Pups with Parent' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	9.373	47.225	<0.001
RESIDUAL	164	0.198		

Position of Father:	NOF	FLE	FRI
Means:	0.448	0.700	1.248
	(18.76)	(41.50)	(89.94)

Table 6.29 Analysis of Variance of 'Pups, Suckling' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	1.381	9.096	<0.001
RESIDUAL	164	0.152		

Position of Father:	NOF	FLE	FRI
Means:	0.223	0.405	0.536
	(4.89)	(15.52)	(26.08)

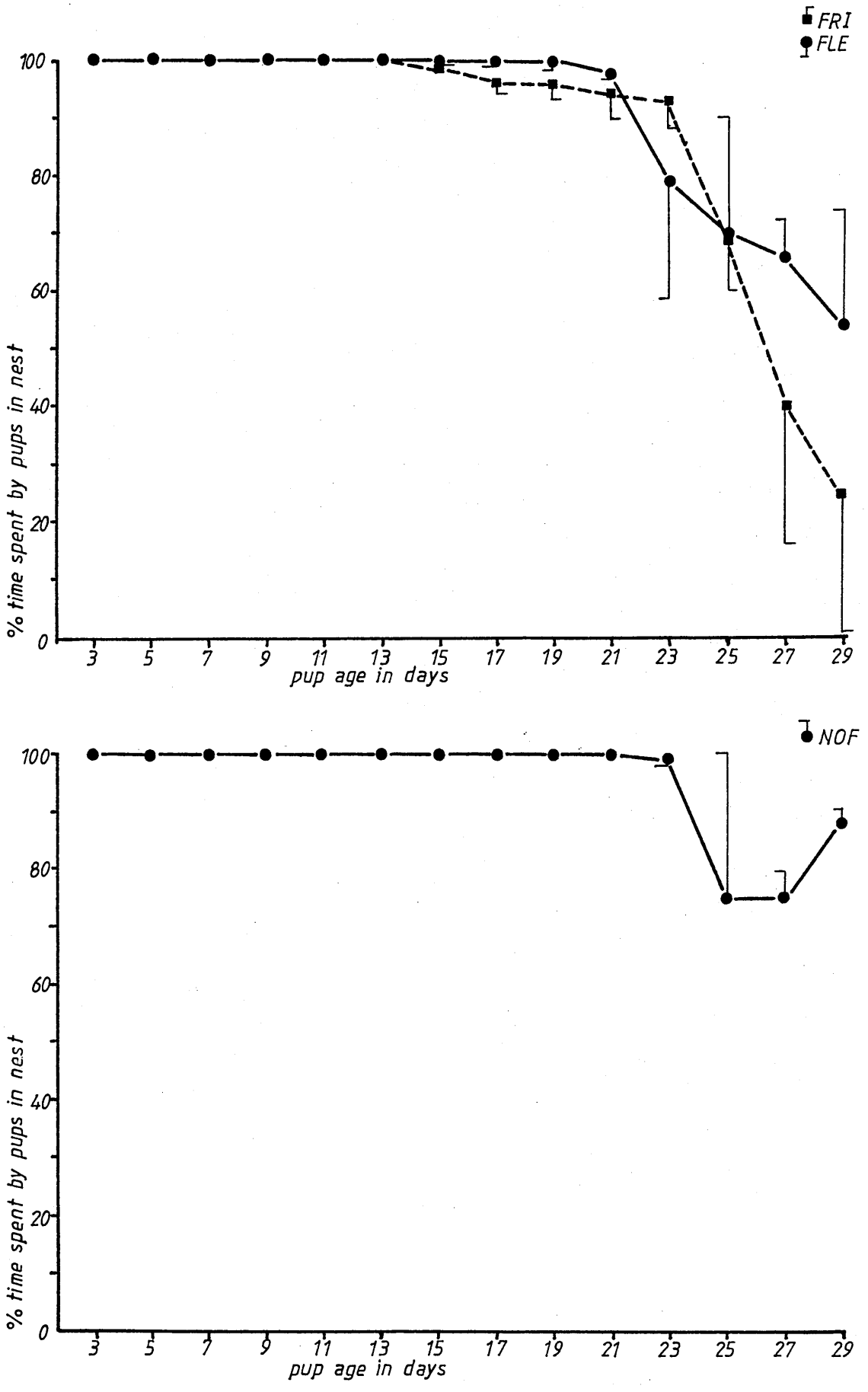


Fig. 6.27 Mean percentage of instantaneous scans of pups in nest. Standard errors are indicated.

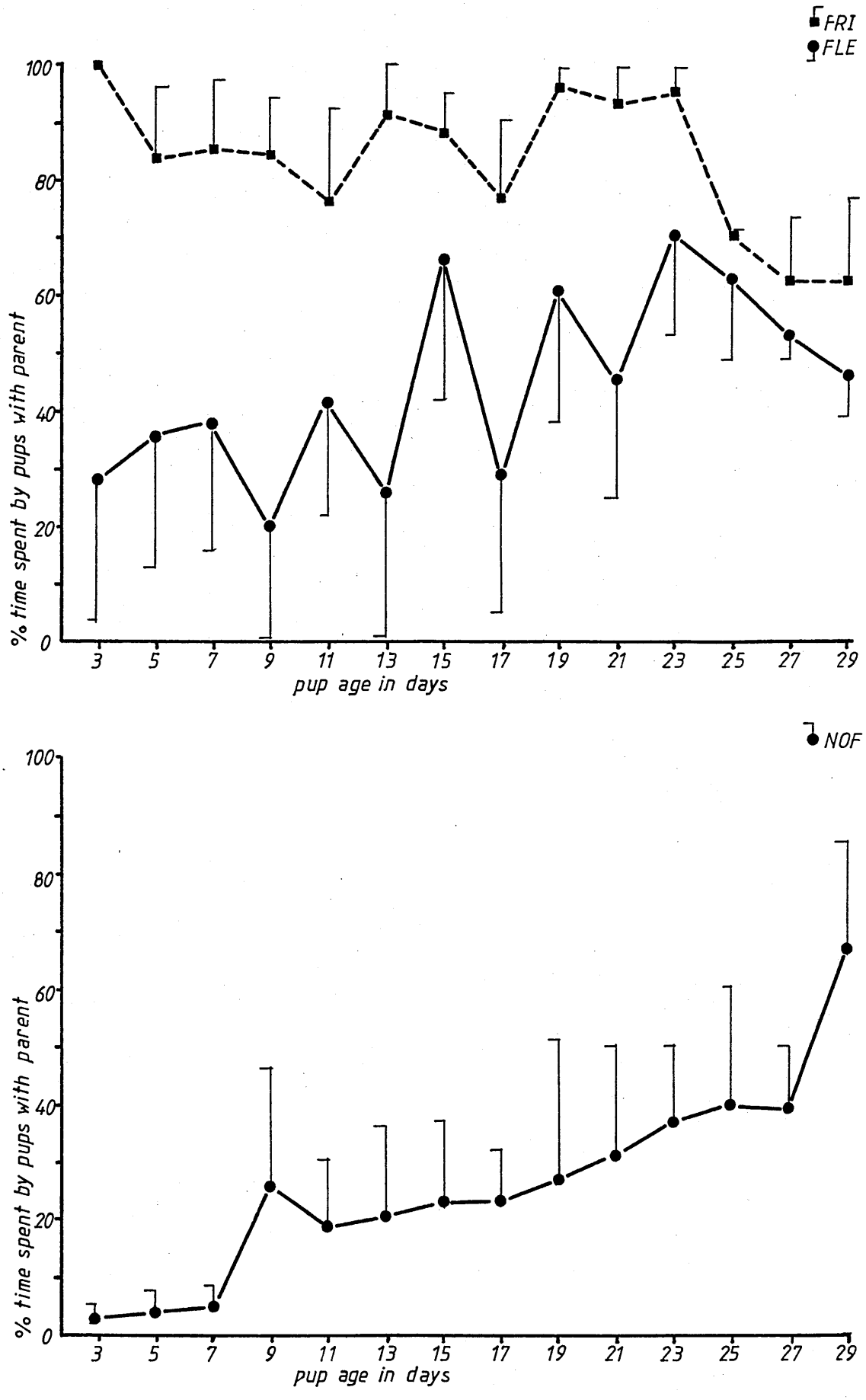


Fig. 6.28 *Mean percentage of instantaneous scans of pups with parent. Standard errors are indicated.*

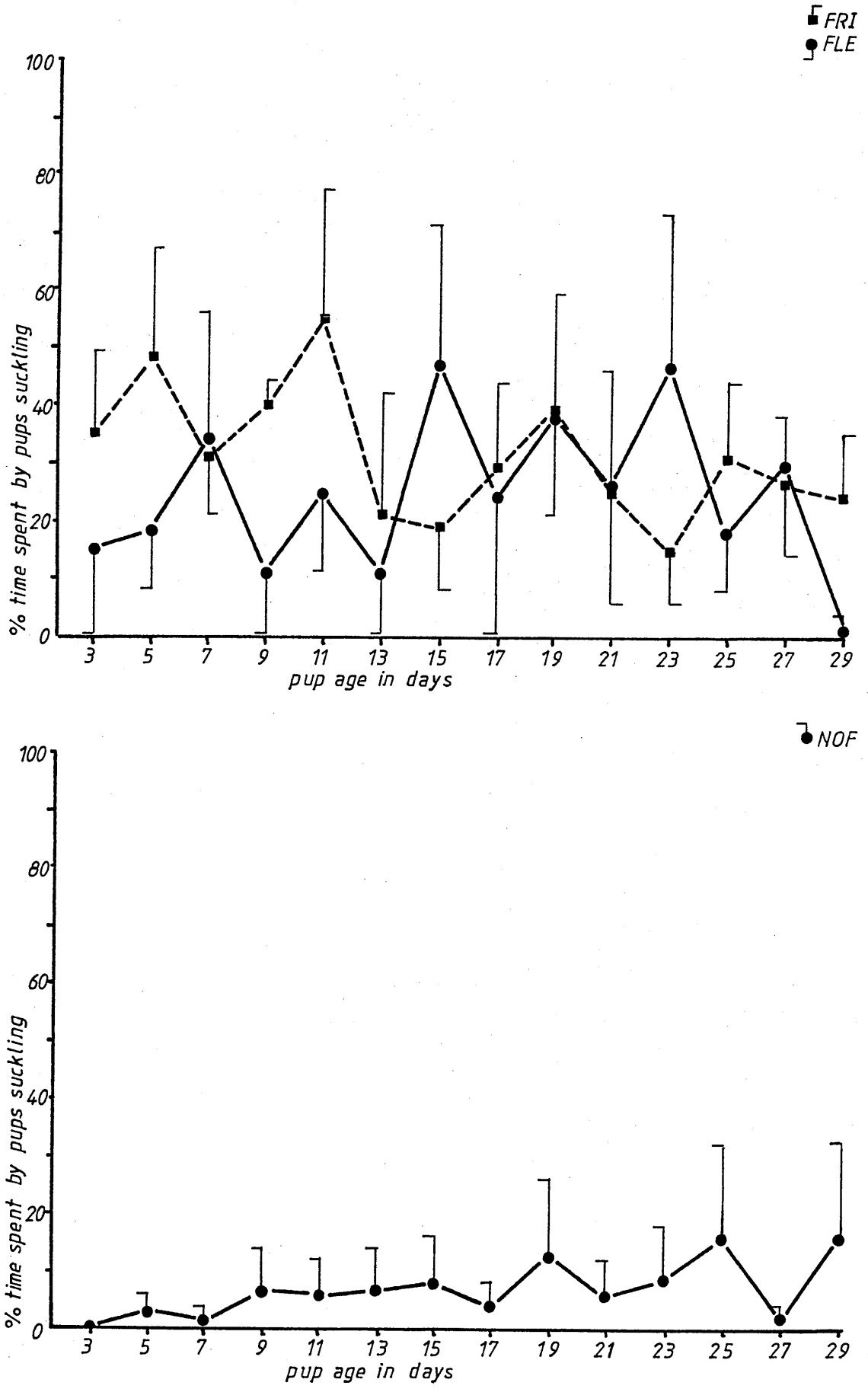


Fig. 6.29 *Mean percentage of instantaneous scans of pups suckling. Standard errors are indicated.*

Table 6.30 Analysis of Variance of 'Pups Eating Solid Food' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.079	3.379	<0.05
RESIDUAL	164	0.023		

Position of Father:	NOF	FLE	FRI
Means:	0.046	0.104	0.116
	(0.21)	(1.08)	(1.34)

Table 6.31 Analysis of Variance of 'Pups, Locomotor Behaviour' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.028	1.928	NS
RESIDUAL	164	0.015		

Table 6.32 Analysis of Variance of 'Pups, Exploratory Behaviour' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.031	3.674	<0.05
RESIDUAL	164	0.008		

Position of Father:	FLE	NOF	FRI
Means:	0.114	0.129	0.160
	(1.29)	(1.65)	(2.54)

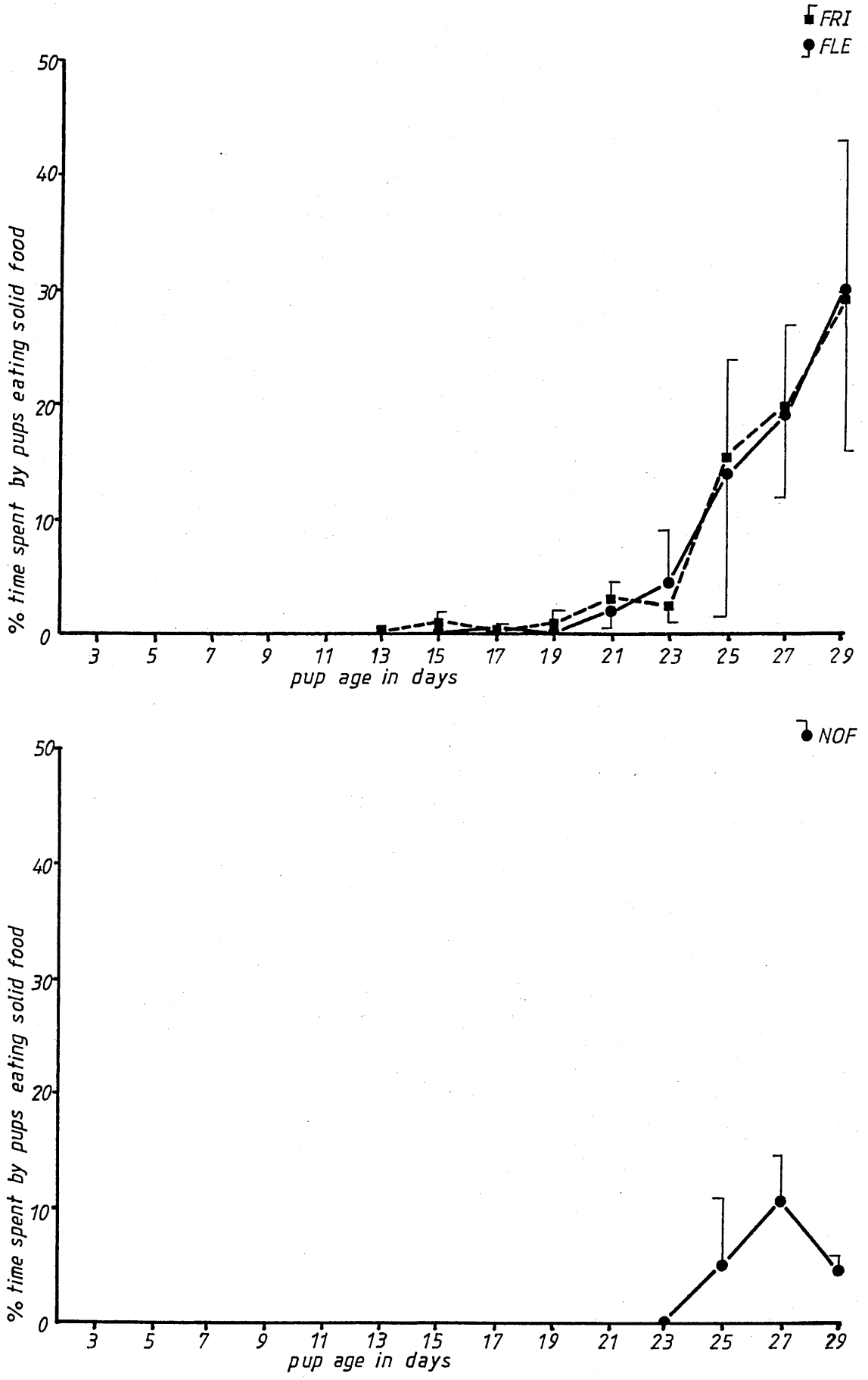


Fig. 6.30 Mean percentage of instantaneous scans of pups eating solid food. Standard errors are indicated.

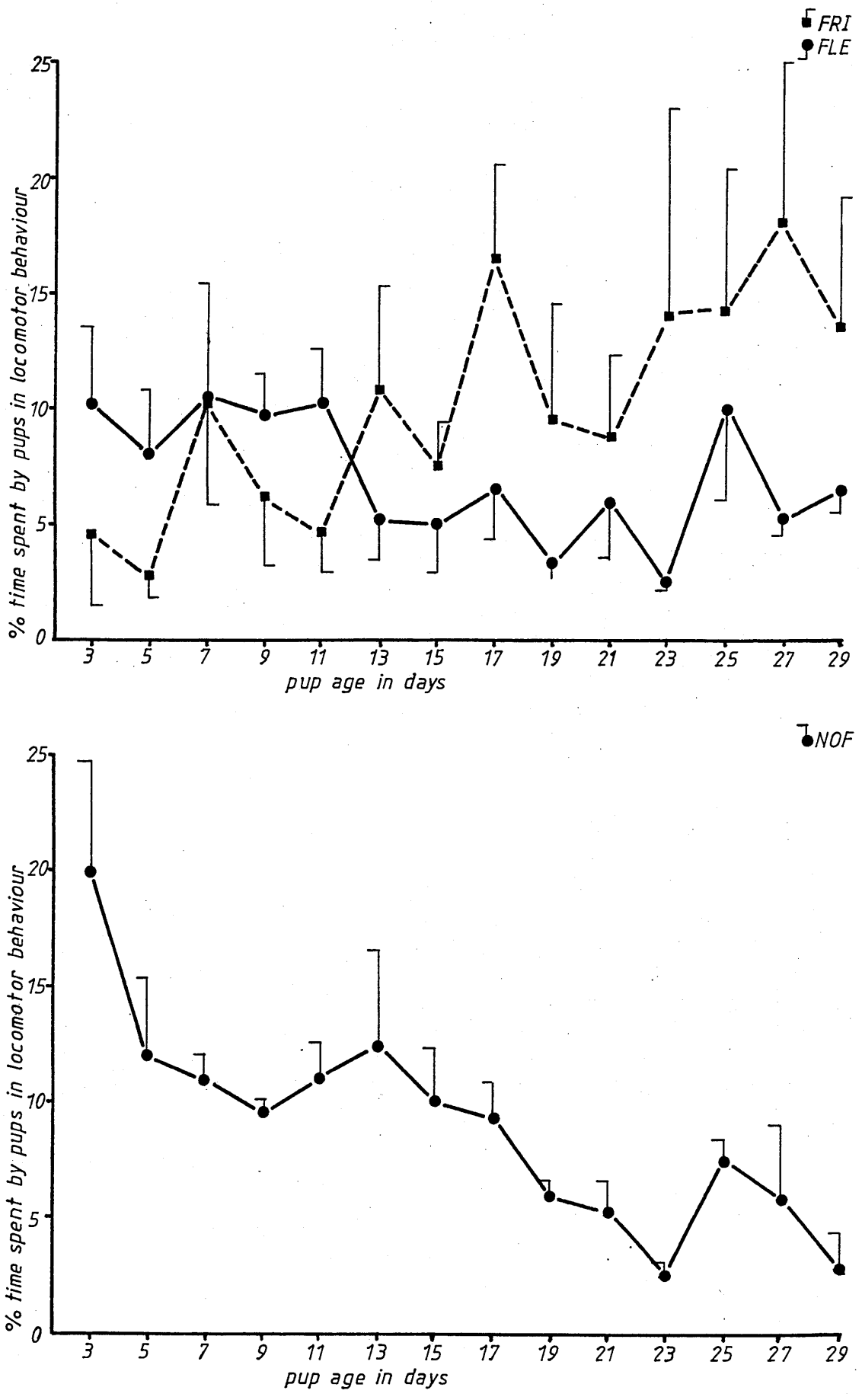


Fig.6.31 *Mean percentage of instantaneous scans of pups exhibiting locomotor behaviour. Standard errors are indicated.*

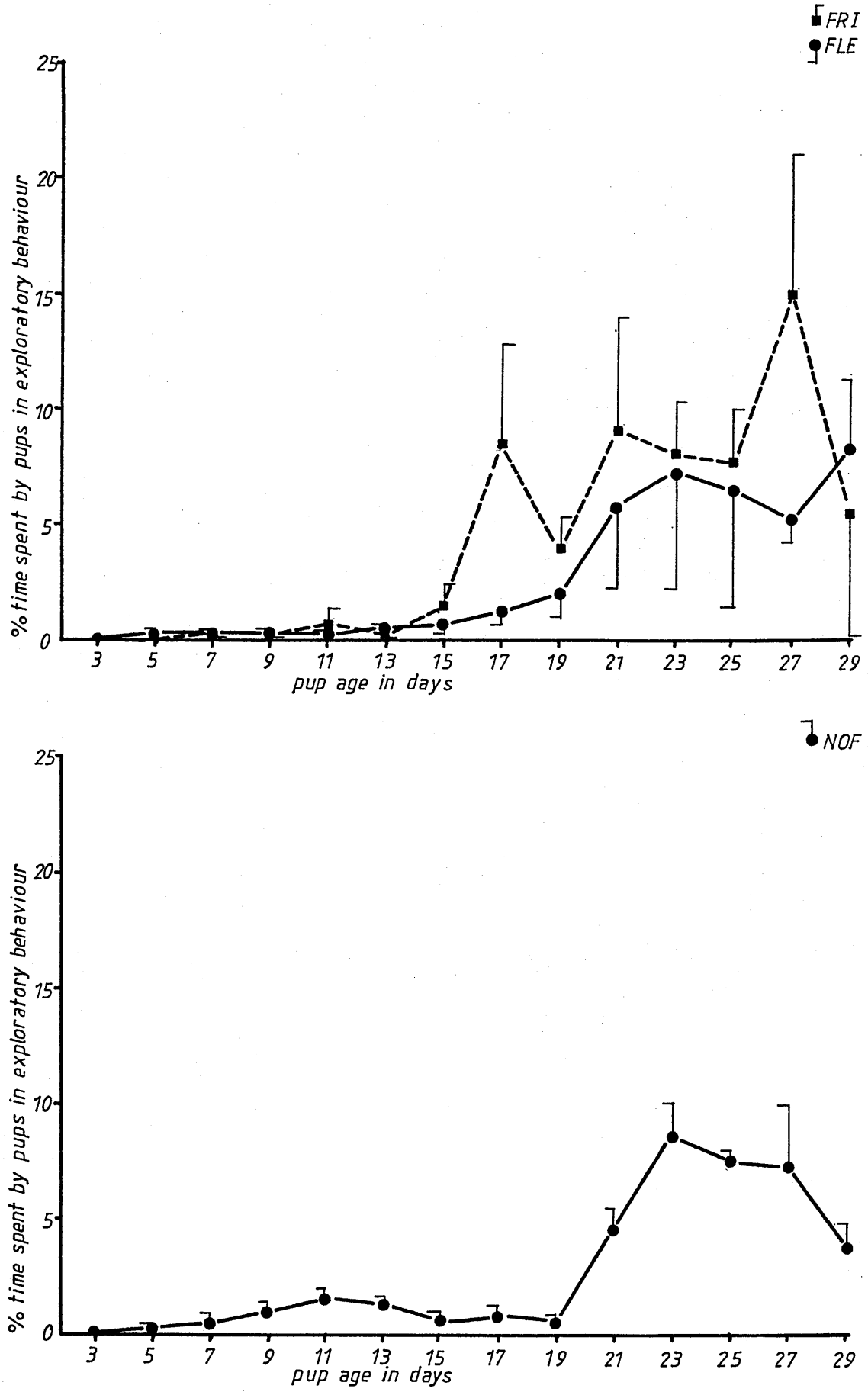


Fig. 6.32 *Mean percentage of instantaneous scans of pups exhibiting exploratory behaviour. Standard errors are indicated.*

Table 6.33 Analysis of Variance of 'Pups, Grooming' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.003	0.259	NS
RESIDUAL	164	0.010		

Table 6.34 Analysis of Variance of 'Pups in Left Compartment' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.071	4.282	<0.05
RESIDUAL	164	0.017		

Position of Father:	NOF	FRI	FLE
Means:	0.003	0.033	0.074
	(0.0009)	(0.11)	(0.54)

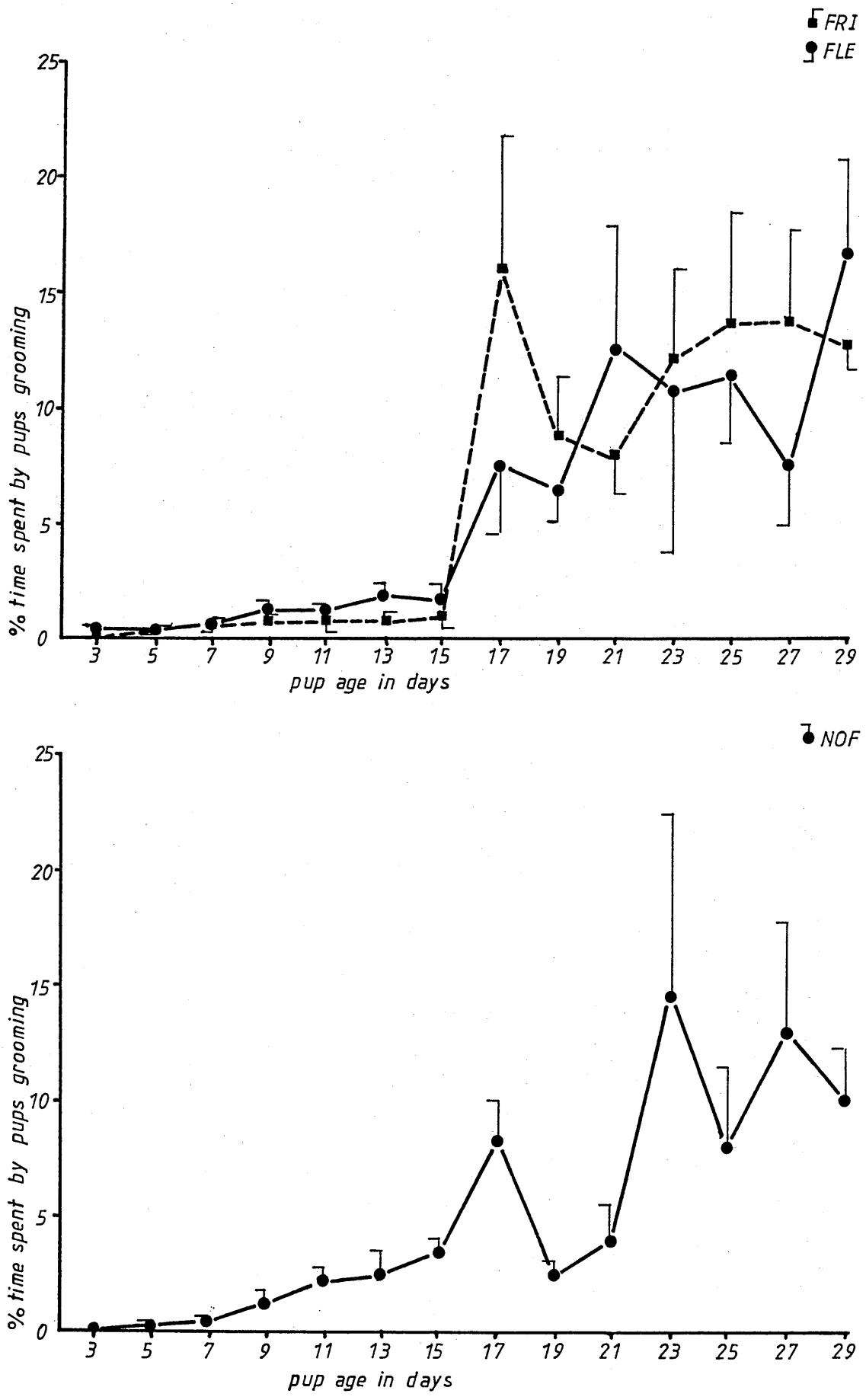


Fig.6.33 *Mean percentage of instantaneous scans of pups grooming. Standard errors are indicated.*

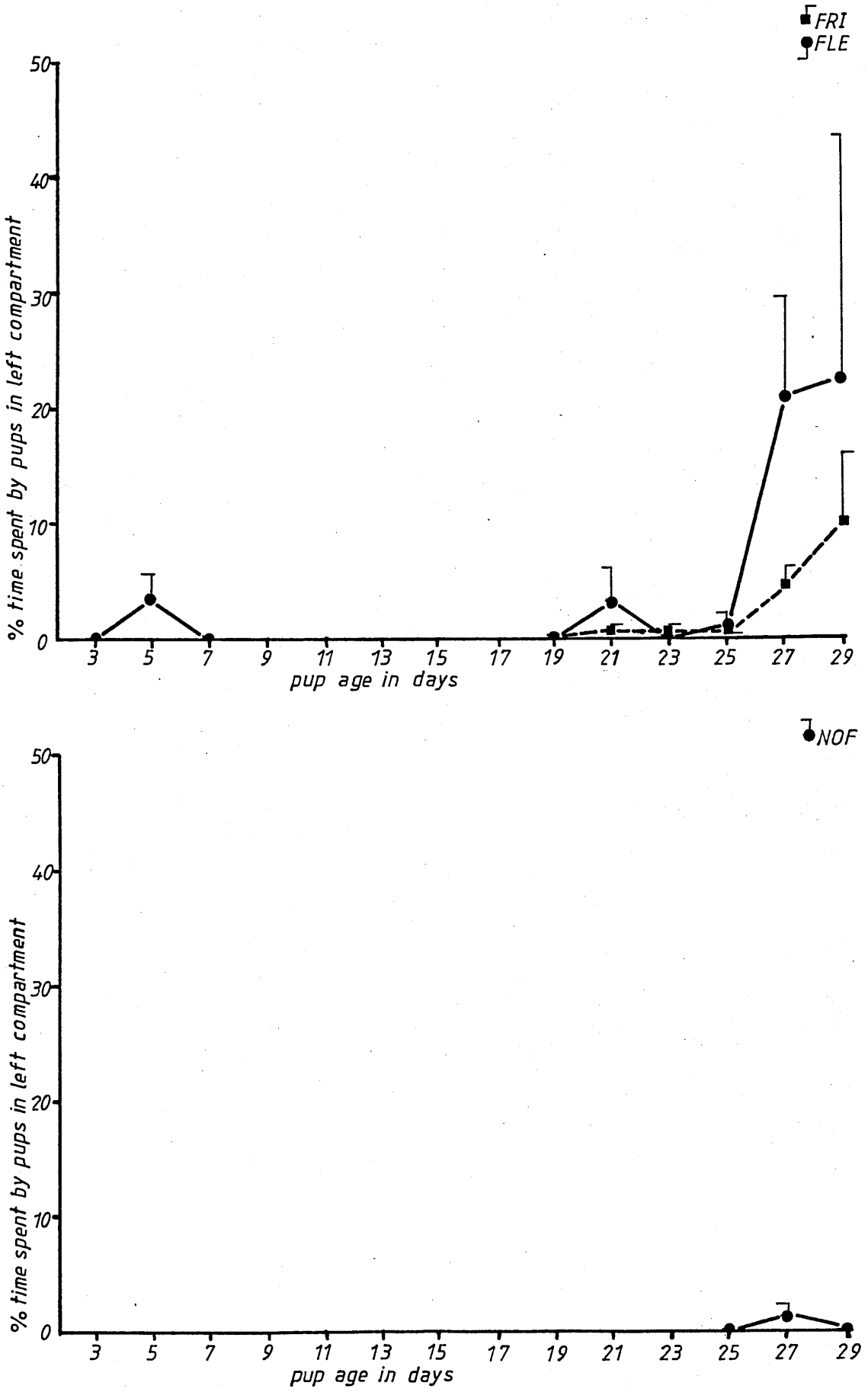


Fig. 6.34 *Mean percentage of instantaneous scans of pups in left compartment. Standard errors are indicated.*

Table 6.35 Analysis of Variance of Open Field 'Peripheral Compartment' Ambulation Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	9485.08	3.87	NS
RESIDUAL	9	2446.39		

Table 6.36 Analysis of Variance of Open Field 'Inner Compartment' Ambulation Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	1092.00	5.38	< 0.05
RESIDUAL	9	202.70		

Position of Father:	FLE	NOF	FRI
Means:	6.25	33.25	36.25

Table 6.37 Analysis of Variance of Open Field 'Rearing' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	133.08	0.82	NS
RESIDUAL	9	160.97		

Table 6.38 Analysis of Variance of Open Field 'Latency to Defaecation' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father:	2	1.08	12.9	< 0.01
RESIDUAL	9	0.08		

Position of Father	FLE	NOF	FRI
Means:	1.00	1.25	2.00

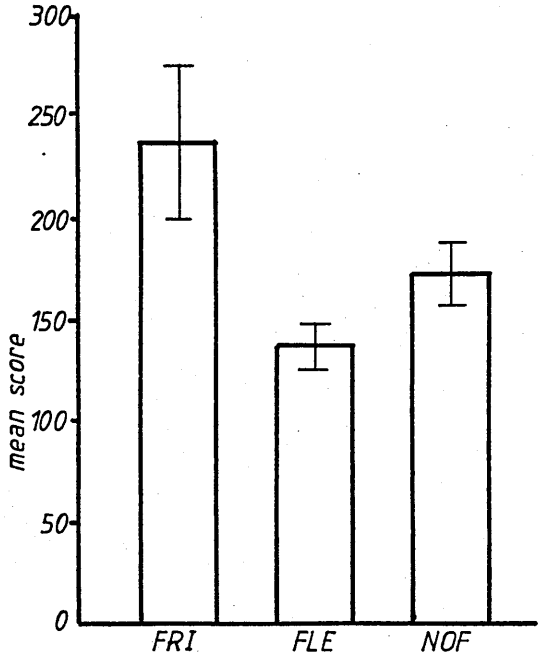


Fig. 6.35 Mean scores for open field peripheral compartment ambulation.
Standard errors are indicated.

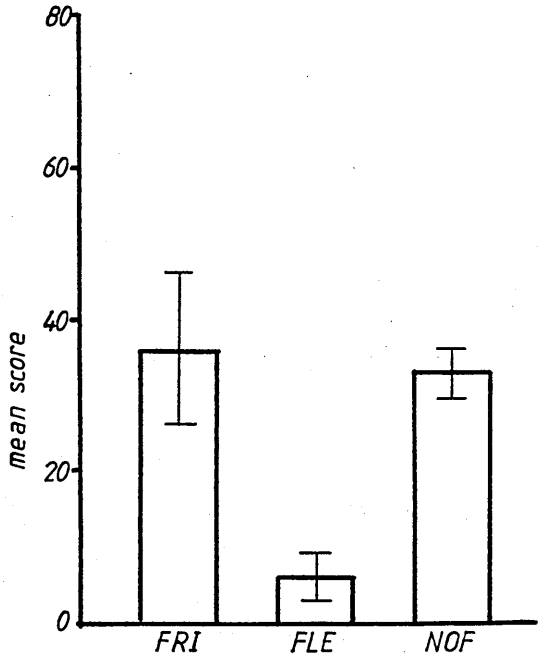


Fig. 6.36 Mean scores for open field inner compartment.
Standard errors are indicated.

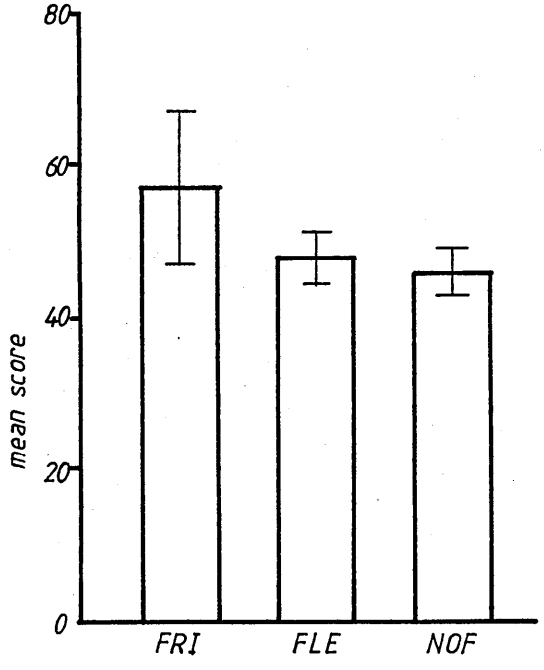


Fig. 6.37 Mean scores for open field rearing.
Standard errors are indicated.

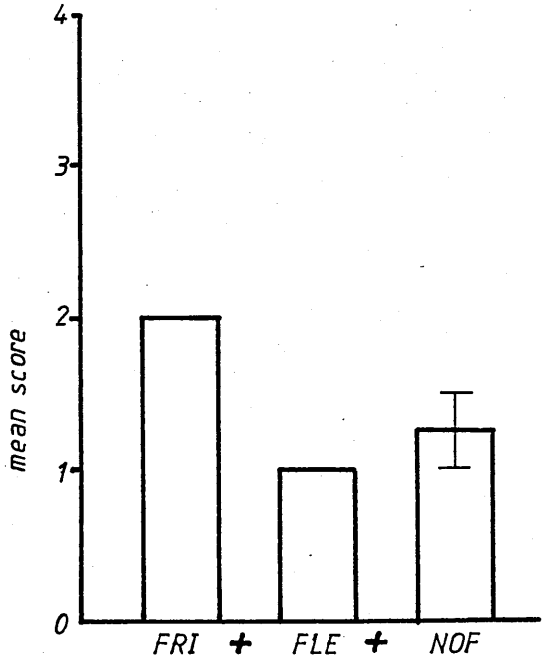


Fig. 6.38 Mean scores for open field latency to defaecation.
Standard errors are indicated.

+ Standard error = 0

Table 6.39 Analysis of Variance of Open Field 'Defaecation' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	2.33	1.23	NS
RESIDUAL	9	1.89		

Table 6.40 Analysis of Variance of Open Field 'Latency to Urination' Scores with Scheffé Test Results

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.75	8.94	<0.01
RESIDUAL	9	0.08		

Position of Father:	NOF	FLE	FRI
Means:	2.25	3.00	3.00

Table 6.41 Analysis of Variance of Open Field 'Urination' Scores

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	0.33	2.98	NS
RESIDUAL	9	0.11		

Table 6.42 Analysis of Variance of Body Weight Data

SOURCE OF VARIANCE	df	MS	F	P
MAIN EFFECT				
Position of Father	2	2.684	0.76	NS
RESIDUAL	9	3.527		

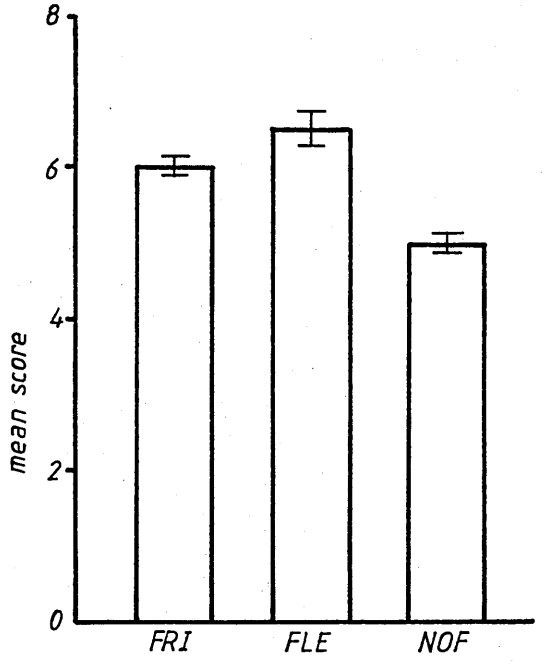


Fig. 6.39 Mean scores for open field defaecation.
Standard errors are indicated.

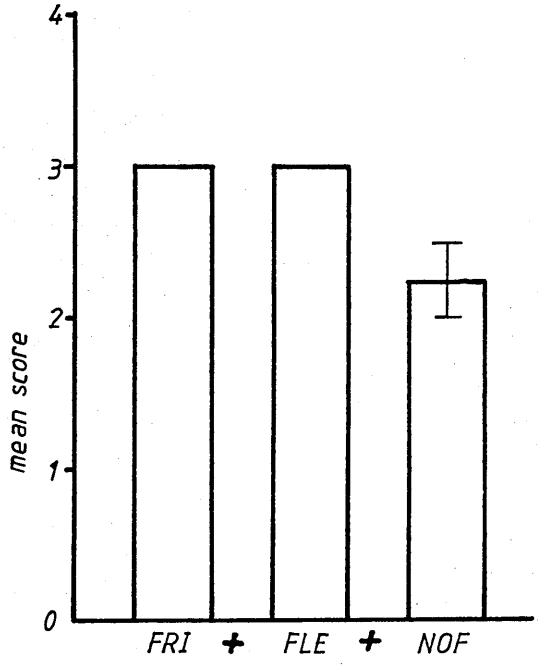


Fig. 6.40 Mean scores for open field latency to urination.
Standard errors are indicated.

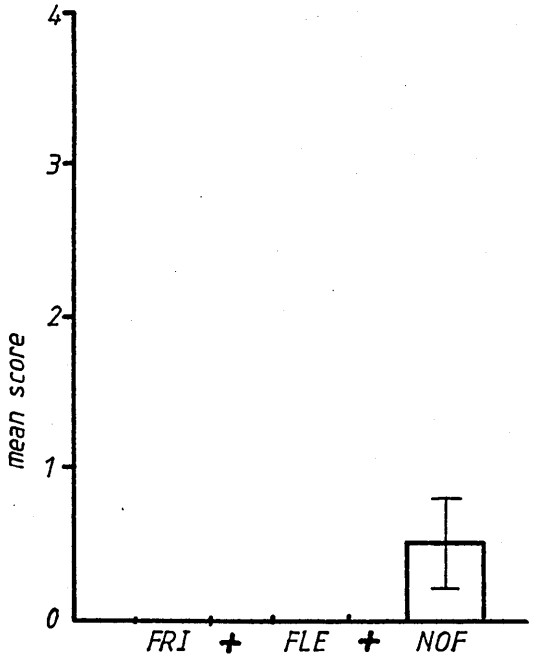


Fig. 6.41 Mean scores for open field urination.
Standard errors are indicated.

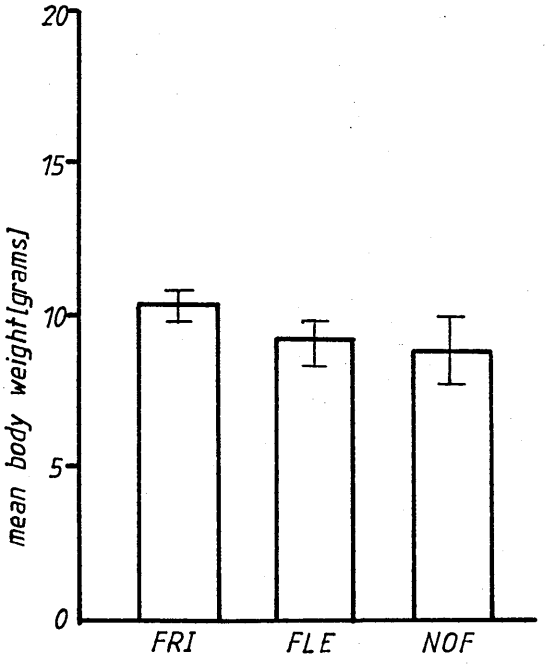


Fig. 6.42 Mean body weights [in grams]
Standard errors are indicated.

\+ Standard error = 0

Table 6.c Summary of Significant Differences in the Results From The Split Cage Experiment

MEASURE	SIGNIFICANT DIFFERENCE	ANOVA P VALUE
Mother in Nest	NOF < FLE	< 0.001
	NOF < FRI	< 0.001
	FLE < FRI	< 0.001
Mother, Nest Construction	NOF < FLE	< 0.05
	NOF < FRI	< 0.05
Mother, Non-Caretaking	FRI < FLE	< 0.001
	FRI < NOF	< 0.001
Father in Nest	FLE < FRI	< 0.001
Father, Stimulating Pups	FLE < FRI	< 0.001
Father, Non-Caretaking	FRI < FLE	< 0.001
Parents in Nest	M ONLY < M & F	< 0.001
	F ONLY < M & F	< 0.001
Pups in Nest	FRI < NOF	< 0.05
Pups with Parent	NOF < FLE	< 0.001
	NOF < FRI	< 0.001
	FLE < FRI	< 0.001
Pups, Suckling	NOF < FRI	< 0.001
Pups Eating Solid Food	NOF < FRI	< 0.05
Pups, Exploratory Behaviour	FLE < FRI	< 0.05
Pups in Left Compartment	NOF < FLE	< 0.05
Inner Compartment Ambulation	FLE < FRI	< 0.05
Latency to Defaecation	FLE < FRI	< 0.01
	NOF < FRI	< 0.01
Latency to Urination	NOF < FLE	< 0.01
	NOF < FRI	< 0.01

'FRI', 'FLE' and 'NOF' refer respectively to the 'father on the right', the 'father on the left', and the 'no father' conditions. 'M' and 'F' refer respectively to 'mother' and 'father'.

Discussion

Table 6.c shows that a large number of significant differences was found in the results from the Split Cage experiment. Some of these results were predictable consequences of the experimental design, particularly those concerning the father's behaviour in the left hand compartment. Other results are more valuable in helping to establish routes of influence between the father and the offspring.

Maternal Behaviour. It has already been suggested that if the maternal mediation route of paternal influence is operating, then the mother's behaviour would be affected by the presence of the father. This is clearly seen to be the case, because out of the four maternal activities measured, three were significantly affected by the position of the father; namely, 'mother in nest', 'mother, nest construction', and 'mother, non-caretaking activity'. An overall pattern is apparent from these three measures affected by the father. The more the mother was exposed to the father, the more caretaking behaviour she exhibited. This is especially exemplified by the 'mother in nest' analysis (Fig. 6.18), where the FRI mean is significantly greater than the FLE and NOF means and where the FLE mean is significantly greater than the NOF mean (Table 6.18). From this result it would appear that when the father was not housed in the same compartment as the mother, but where auditory and olfactory communication was possible, the mother was still affected by the father. The father's influence served to trigger or release a greater amount of nest attendance in the mother.

It might be expected that when the father was present with the litter, some relief from the caretaking burden would be afforded the mother. Instead the opposite effect appeared to operate. The response

of the mother to spend more time in the nest when influenced by the father's presence could have been a protective gesture, related to the possibility that the father might harm the pups. Such a reason may seem unwarranted, however, when the strong caretaking role of the father is considered.

It is more likely that the father does share the caretaking functions of the mother and relieves the mother of some caretaking duties. This would mean that when the father was absent, the mother's resulting caretaking load would be very much greater than when he was present. The mother's response to this increased load appears to be to spend more time away from the litter. This notion of litter or nest aversion again raises the suggestion of maternal fatigue and its associated inhibition of the display of maternal behaviour (Seitz 1958). It should be noticed, however, that the NOF mothers, which were the group to spend most time out of the nest, were the group to spend most time involved in non-caretaking activities (Fig. 6.21). If fatigue implies physical exhaustion, then it is an inappropriate term to use for the NOF mothers. Instead of equating nest avoidance with rest, it needs to be associated with activities unrelated to caretaking.

Elwood & Broom (1978) suggested that increased bar gnawing in gerbil mothers rearing a litter in the absence of the father was an expression of disturbance caused by the litter. It is likely that the Split Cage mothers left the nest because they were disturbed by the stimulus characteristics of the litter. Elwood & Broom (1978) proposed pup ultrasonic calling as a likely agency evoking this maternal effect and this could also be applicable in my own study. The father's presence would tend to reduce the production of cold-induced ultrasounds (Elwood 1979b; Okon 1970a) by insulating the litter

and reducing heat loss. In the absence of the father then, there would be an increase in the production of ultrasounds. Although Noirot (1972b) found that cold-induced ultrasounds constituted an attractant to the mother, the reaction of the mother may be affected by her current state. Bell (1974) claimed that the reaction to ultrasounds depends on the extent of arousal in the recipient. It is possible that a mother disturbed by the litter will respond to this disturbance by leaving the nest. Bell et al. (1974) found that the persistent production of cold-induced ultrasounds agitated rat mothers and prevented the display of normal maternal behaviour. Why is it, however, that a remote father effect also seemed to be operating ? The FLE mothers spent significantly more time in the nest than the NOF mothers, yet the father was absent from the nest in the mother compartment in both cases. The father could only have influenced the physical environment of the litter; for example, in insulating the litter from heat loss; by actually being present in the same compartment as the litter, but other sensory cues from the father could have affected maternal or pup behaviour. It is possible that the mother associated the sounds or odours of the father with actual paternal presence and behaved in a way which resembled the normal maternal behaviour which was displayed when the father was present. This would explain why there was more maternal nest attendance in the FLE mothers than the NOF. The pups likewise may have registered paternal presence through auditory and olfactory communication from the father, and through consequent behavioural changes, may have provided a different stimulus output to the mother. Such changes in the litter caused by the father would very likely evoke changed maternal behaviour.

For the highest maternal nest attendance scores, however, the father needed to be actually present with the mother and litter. This may indicate that a combination of mechanisms were causing the father's effect on maternal behaviour. Whereas mechanisms involving the father's direct presence are more likely and more plausible, it is possible that an auditory/olfactory mechanism was also operating. Parental ultrasound production in rodents has received some attention (Okon 1970b; Sales 1972b) as has adult male rodent odour production (Stoddart 1976; Wilson 1970). Scott & Pfaff (1970) found that female mice spent more time sniffing tubes containing urine from normal males than tubes containing urine from castrated males, showing a female interest in male odours. Further work investigating inter-parent auditory/olfactory communication would now be most profitable.

Regardless of the mechanism, the effect of the father's presence was to cause the offspring to have more contact with the mother than when the father was absent. This increased contact would represent a greater amount of tactile stimulation, thermal insulation and suckling opportunity for the developing pups, which may have been partly responsible for changes seen in pup behaviour when reared in the presence of the father in the main experiment.

An increased display of maternal behaviour in the presence of the father was also extended to nest construction (Fig. 6.20), where the FRI mean score was significantly greater than the NOF mean score. The mother's non-caretaking activity scores also reflect this same pattern, since the FRI mean was significantly smaller than both the FLE and the NOF means (Fig. 6.21). An examination of the suckling means for the pups reveals the same pattern (Fig. 6.29). The FRI mean was significantly greater than the NOF mean, which indicates that pups

were able to take advantage of the extra suckling opportunity provided by the increased nest attendance of the mother. With the mother's nest construction, the mother's non-caretaking behaviour, and the pups' suckling analyses, the FLE means were larger than the NOF means. This would tend to lend support to the claim for the existence of auditory or olfactory communication between the father and the mother housed in different compartments.

Increased nest construction in the FRI mothers compared to the NOF mothers would work in the direction of minimizing heat loss from the litter. Sealander (1952) found that nest building increased the toleration of extremely low temperatures in deer mice. Fig. 6.20 shows that nest building is more likely at an increased pup age in the FRI mothers, compared to the other two groups. This may not, however, be a direct response to paternal influence, but instead a response to offspring stimulation which has, itself, been altered by the father's presence. One possible candidate for this would be increased ultrasound production in pups exposed to the father. This does not necessarily contradict the earlier suggestion that the father's presence would serve to reduce cold-induced ultrasounds. Noirot (1972b) and Okon (1970a, 1970b) referred to two types of ultrasounds produced by mouse pups. Cold-induced ultrasounds were seen to be different from ultrasounds caused by the reception of tactile stimulation and different maternal responses were evoked by each. Paternal presence would tend to reduce cold-induced ultrasound production and increase those ultrasounds produced by tactile stimulation. It is, however, the former type which appears to evoke maternal nest building in rodents (Noirot 1972b), and this makes it unlikely that the increased nest construction by the mother resulted from her response to ultrasonic calls emitted by the pups when they were disturbed by the father. Bell et al. (1971) reported that deer mice which were

handled during their pre-weaning period emitted very obvious ultrasounds which elicited increased maternal behaviour. This, however, may have been caused by cooling, rather than by tactile stimulation. Another aspect of the results which throws doubt on the ultrasound suggestion is that maternal behaviour differences between the three groups persist for almost the entire recording period (Figs. 6.18, 6.20 and 6.21). Ultrasound production by pups is unlikely to account for these differences at an older pup age (Noirot 1974; Okon 1970b; Richards 1967).

The above discussion has considered the possibility that the father's effect on the mother was mediated by changed pup behaviour, rather than by a direct effect on the mother. Ressler (1962) claimed that mouse mothers are responsive to subtle characteristics of the offspring. The idea that paternal effects on maternal behaviour could be mediated by the changed stimulus characteristics of the litter, as proposed by Elwood & Broom (1978) in gerbils, is still plausible in laboratory mice but the sensory modality remains to be investigated. Several other reports support the notion that maternal behaviour is affected by the stimulus characteristics of the litter, including Barnett & Burn (1966, 1967), Priestnall (1973a), Richards (1966a), Rosenblatt & Lehrman (1963) and Young (1965).

It would follow that if the mother was spending a greater proportion of time involved in caretaking activities, then less time would be available for self-directed behaviour such as eating, self-grooming and exploration. This is verified by the mother's non-caretaking activity data throughout the entire recording period (Fig. 6.21).

An expected relationship is observed between the 'pup suckling' scores and the 'mother in nest' scores; especially at a younger pup age when the litter was restricted to the nest (compare Figs. 6.18 and 6.29). Lower scores, however, were obtained for pup suckling in the NOF group than would have been anticipated on the basis of data obtained from the main experiment, where no significant difference was found due to the paternal presence factor. The suckling scores were limited by the amount of time the mother spent in the nest, until the pups were able to leave the nest and approach the mother themselves. Design differences between the main experiment and the Split Cage experiment include a different cage unit and an earlier onset of the recording period. These differences may account for differences between the suckling scores for the two experiments. The characteristics of the Split Cage design may have, in some way, caused the deflated NOF maternal nest attendance and suckling scores. The greater visual access presented to a human observer with this cage, may have evoked a higher anxiety level in the mother, which in turn could have modified maternal behaviour. The other consideration, of recording time, is important. Behaviour between day 3 and day 9 was not recorded in the main experiment, yet this represented the pup age when the greatest difference between the three groups was found in the Split Cage experiment. Even though the suckling time of the NOF pups was reduced in comparison with the FRI pups, there was no corresponding body weight reduction on day 30. From this measure, therefore, there is no indication that a permanent inhibition of development was caused by the smaller amount of time spent in suckling. An examination of Fig. 6.30 reveals that decreased suckling was not compensated for by increased ingestion of solid food in the NOF pups.

No significant differences were found between the three groups for maternal stimulation of pups. This activity area included such specific activities as licking and grooming which are considered to be expressions of maternal behaviour (Hatton & Meyer 1973; Leblond 1940; Reisbick et al. 1975). Whereas the father's presence affected maternal behaviour, it did not affect all types of it. This highlights the need to measure several aspects of maternal behaviour rather than to assume that any one will be typical of them all. Grota & Ader (1969), for example, measured the time that a lactating rat spent with its litter as an indication of maternal behaviour. Hinde (1959) cautioned against the indiscriminate use of unitary concepts of drive which propose that different behavioural characteristics depend on the same features of an underlying mechanism. Elwood (1979a) commented that maternal behaviour was not a unitary process in gerbils since there was a negative correlation between some different maternal activities. Slotnick (1967) concluded that the term 'maternal behaviour' did not represent a unitary behavioural process, after studying caretaking behaviour in female rats.

In summary, differences in maternal behaviour due to partial and total paternal presence are demonstrated in the 'mother in nest', 'mother, nest construction' and 'mother, non-caretaking activity' data. Elwood & Broom (1978) found that, in gerbils, in the presence of the father, the mother sniffed the pups less and nest built less. This indicates that there was less maternal behaviour exhibited in the father's presence. There were, however, no significant differences between the amounts of time the mother spent in the nest and in contact with the pups when the father was present, compared to when he was absent. Dudley (1974b) found that the mother California mouse spent more time in the nest when the father was not present; the very opposite

to the result found in my own experiment. Such a difference may have been caused by the species difference in the two experiments. When Priestnall & Young (1978) investigated the effect of the adult male on maternal behaviour in laboratory mice, however, they concluded that the male had very little influence on the female's caretaking behaviour. There were no significant differences between mothers housed with or without the male for nursing, retrieving, licking, nest building and time spent in the nest. Two differences between this experiment and the Split Cage experiment, which may account for differences between the two sets of results, are the parity of the mothers and the strain of mouse used. All mothers were primiparous in Priestnall & Young's experiment whereas they were multiparous in my own. They also used the CFLP strain in comparison with the BALB/c strain used in the Split Cage experiment.

Paternal Behaviour. Not surprisingly, the father spent significantly more time in the nest with the pups, more time stimulating pups and less time involved in non-caretaking behaviour when housed in the right hand compartment with the mother and litter, compared to when the father was housed in the left hand compartment. All of these results were determined by the fact that pups spent more time in the right hand compartment than the left. A number of interesting points emerge, nevertheless, from the records of the father's activities. Figs. 6.22 and 6.23 reveal a sudden emergence of caretaking behaviour in the FLE mean scores on days 5/6. This was caused by the father managing to pull a pup through one of the pup filter holes from the mother compartment and occurred in two of the four replicates at this time. At no other time was this observed because the chance of a pup being close to a pup filter hole was remote. In most cases the mother established the nest well away from the pup

filter divider. On one of the occasions when this did occur, the father and the mother were both attempting to pull the pup into their own compartment. Eventually the father was successful and retrieved the pup to his own nest. In the nest, the father adopted a posture characteristic of the mother's nursing position and remained there for some time. This again demonstrates the readiness of the father to display caretaking behaviour. Elwood (1957b) explained that a gerbil father, if prevented from having access to the litter by the mother, may remove young pups and build his own nest. Noirot (1969b) reported that adult laboratory mice retrieved as readily as females did.

Once FLE pups began to spend time in the father compartment, the father immediately displayed caretaking behaviour. Nest construction was increased and there was a display of nest attendance and pup stimulation (Figs. 6.34 and 6.22 to 6.24). Although the FLE father was deprived of contact with the pups for a number of days soon after their birth, he would have been subjected to a barrage of ultrasounds and odours during this period. This sensory input may have served to maintain the father's readiness to perform in a caretaking fashion when the opportunity was presented. Records of the father's behaviour, then, serve more to demonstrate the active caretaking role of the father than to throw light on the likelihood of the maternal mediation route for the father's influence.

An examination of the behaviour of the FRI fathers shows again that caretaking behaviour is readily displayed, even in the presence of the mother. In fact a higher overall mean value was obtained for the father in comparison with the mother, for stimulating pups (Figs. 6.19 and 6.23). (This does not demonstrate a paternal mediation route for maternal behaviour effects on pups, since the father's caretaking

behaviour was just as evident in the absence of the mother). Priestnall & Young (1978) found that male laboratory mice displayed as much caretaking behaviour as females.

The analysis conducted on the time spent by FRI parents in the nest revealed that both parents were in the nest together for a significantly greater proportion of the time than either the father or the mother were in the nest alone. For the times when only one parent was in the nest, there was an inverse relationship between 'father only' and 'mother only' scores (Fig. 6.26). No significant difference was found between the amount of time spent by the mother alone and the father alone, in the nest. Elwood (1983) commented on the time spent in the nest by rodent fathers. For the laboratory mouse, he reported that the adult male spends increasingly more time in the nest during the first half of the pre-weaning period and spends more time than the female during the second half. This description resembles my own data as revealed by a comparison of Figs. 6.18 and 6.22. Dudley (1974b) found in California mice that each parent spent more time in the nest when the other parent was out of the nest, and that the father spent as much time in the nest as the mother. Waring & Perper (1980) obtained similar results with gerbils. Once again certain similarities, but also differences, exist between the subject species. Spencer-Booth (1970) reported that fathers in several rodent species were kept away from the nest by the mother during the first few postpartum days. This was especially true in the dormouse (Glis glis) and in Peromyscus spp. Elwood (1975b) also observed father exclusion from the nest by the mother in gerbils. This does not appear to be the case in the laboratory mouse since the FRI father was able to display caretaking behaviour from the beginning of the recording period which was at a very early pup age.

Pup Behaviour. The 'pups with parent' data show that FRI pups have a significantly greater overall mean than FLE pups and that FLE pups have a significantly greater overall mean than NOF pups (Table 6.28 and Fig 6.28). This latter result is not surprising, given the design of the experiment. Since both parents display caretaking behaviour, pups receiving the most parental contact would be likely to receive most caretaking behaviour and to reflect this in their developmental behaviour. An examination of the other pup activities reveals the extent to which behaviour and development was affected by the father's position.

FRI pups spent more time out of the nest than NOF pups (Table 6.27) and also began to leave the nest earlier than the other two groups (Fig. 6.27). This is consistent with the main experiment where pups, raised by multiparous mothers and in the presence of the father, spent less time in the nest than other groups. Since pups spend less time in the nest with increasing pup age and development, this result may indicate that pups having most access to the father were developing at a faster rate. Also in common with the main experiment results, the eating solid food scores were higher in the FRI pups than the NOF pups (Table 6.30 and Fig. 6.30). Since the onset of this activity is a developmental milestone, accelerated development may again be indicated by this result.

Returning again to the main experiment for comparison purposes, a higher incidence of exploratory behaviour was associated with the presence of the father. This, again, is reflected in the Split Cage results where the FRI pups spent a greater proportion of time involved in exploratory behaviour than the FLE pups (Table 6.32 and Fig. 6.32).

For the 'pups in the left compartment' result, the FLE pups obtained a significantly greater overall mean score than the NOF pups (Table 6.34). This would indicate that the father constituted an attractant to the pups, perhaps because of the odours produced by the father. Alternatively, the caretaking behaviour provided by the father may have reinforced the pups' behaviour of visiting the father's compartment.

In common, again, with the main experiment, the father's presence did not significantly affect the pup locomotor behaviour scores. An examination of Fig. 6.31, however, reveals an interesting age trend for the FRI and the NOF pups. Whereas pup locomotor scores increase with age in the former, they decline in the latter. This difference would be worthy of closer examination in the future.

Three of the day 30 scores showed significant differences. Open field inner compartment ambulation scores were higher in the FRI subjects than the FLE (Table 6.36). In the main experiment, this activity was involved in a three way interaction where the greatest mean value was obtained by the 7-Pr-F group. The father's presence would appear to be associated with a readiness on the part of the offspring to ambulate in the central area of the field, which requires leaving the walls of the apparatus. An anomalous result is that of the NOF pups which obtained a higher score than the FLE pups for inner compartment ambulation.

Latency to defaecation was significantly greater in the FRI subjects than the FLE or the NOF subjects (Table 6.38). Latency to urination was significantly less in the NOF subjects than the FLE or the FRI subjects (Table 6.40). It would appear that increased exposure

to the father results in increased latency to elimination scores for the subjects. The open field scores provided evidence that the position of the father affected the manner in which the offspring responded to a novel environment.

On the basis of the two previous experiments, it would be expected that body weights would be related to the amount of paternal influence received by the subjects. In all three experiments, the presence of the father was associated with the provision of thermal insulation and tactile stimulation. It was considered that these two aspects of caretaking behaviour could have been responsible for increased body mass of pups at day 30. Although the pups' body weights were greatest in the FRI condition and lowest in the NOF condition (Fig. 6.42), the differences between the three groups were not significant (Table 6.42).

Conclusions

For the maternal mediation argument to hold, the results from the Split Cage experiment would need to show both, that the mother's behaviour was different, and that the pups' behaviour was different, under the three conditions. Both of these sets of differences were apparent in the results. The experiment showed that maternal behaviour was altered by the presence or absence of the father. The father's presence was associated with high 'in nest' and 'nest construction' scores and low 'non-caretaking' scores, for the mother. The most obvious effect was found when the father was present with the mother, but an effect was still apparent when only auditory and olfactory communication between the two parents was possible. The father-induced change in maternal behaviour was likely to affect the

offspring since it involved an increased display of some maternal caretaking activities. The offspring's behaviour was affected; and to add support to the suggestion that the maternal mediation effect contributed to the main experiment results, there was similarity between the offspring results of the main experiment and the Split Cage experiment. In both experiments, the father's presence was associated with elevated 'solid food' and 'exploratory' scores and depressed 'in nest' scores. Also in the Split Cage experiment, the 'with parent', 'suckling', 'inner compartment ambulation', 'latency to defaecation' and 'latency to urination' mean scores were greater in the FRI condition than other conditions.

In addition to demonstrating the existence of a maternal mediation effect, the Split Cage experiment also reinforced a conclusion drawn from the Split Litter experiment, which was that the father laboratory mouse displays an extensive range of caretaking activities and that this must also contribute to the paternal presence effect on mouse pup developmental behaviour.

With few exceptions, there was close consistency between the results of the main experiment, the Split Litter experiment and the Split Cage experiment.

Evidence is provided by the Split Cage experiment for the existence of the maternal mediation route of paternal influence. Together, the Split Litter experiment and the Split Cage experiment, provide support for both paternal effect hypotheses. The father would appear to influence pup development by both a direct route and by a maternal mediation route.

4. Chapter Summary

1. Two experiments were conducted to investigate the possibility that the father affected laboratory mouse pups' behaviour and development by:

   (a) a direct route, and

   (b) a maternal mediation route.

2. The Split Litter experiment subjected members of a litter to a particular parental influence for a length of time each day during the pups' pre-weaning period. The parental influences were exposure to the mother only, exposure to the father only and no exposure to either parent.

3. The Split Litter experiment demonstrated the existence of a direct route. In the absence of the mother, the father displayed a range of caretaking activities very similar to those exhibited by the mother. Of special interest, was the thermal insulation and the tactile stimulation received by the offspring from the father.

4. The measurement of a number of pup activities in the Split Litter experiment revealed that the father's interaction with the pups caused changes in the pups' developmental behaviour. The pup measures affected were 'proximity to parent', 'locomotor behaviour', 'sniffing', 'head-lifting', 'open field defaecation' and body weight.

5. The Split Cage experiment caused pups to be reared in three different conditions regarding the position of the father. In the first situation the father was placed in the same cage compartment as the mother and litter. In the second situation the father was placed in an adjoining cage compartment where only the pups could have access to

him. In the third situation the father was removed completely.

6. In the Split Cage experiment an increased display of behaviour was elicited by the presence of the father. Physical contact with the father was not necessary for this effect on the mother, although it did increase the effect. Since this changed maternal behaviour, due to the presence of the father, appeared in turn to affect the pups, it was suggested that a maternal mediation effect was operating.

7. Some evidence was provided by the Split Cage experiment to support the idea that in this triadic interaction, some inter-animal communication proceeded through auditory or olfactory routes.

8. It was concluded that the laboratory mouse father affected offspring by both :

(a) a direct route, and

(b) a maternal mediation route.

Chapter Seven

GENERAL DISCUSSION AND CONCLUSIONS

Chapter 7

GENERAL DISCUSSION AND CONCLUSIONS

1. Noticeable Points Arising From the Experiments

Looking across the results of the different experiments, certain consistencies are apparent. The most noticeable points to emerge are disscussed below.

(i) The Rate of Development

Three particular factors or groups of factors appeared to increase the rate of development. The offspring in the smaller litters obtained a significantly greater day 30 body weight mean than offspring in larger litters. This is likely to have been caused by the small-litter pups' greater access to parental stimulation than the pups in the larger litters. Brumby (1960), Eisen et al. (1977), Gates (1925) and Priestnall (1972) all found that mouse pups in smaller litters experienced greater weight gains than pups in larger litters.

The interaction between the small litter size and the multiparous mother appeared to accelerate certain aspects of behavioural development. Pups reared under these circumstances spent significantly more time, overall, out of the nest, away from littermates, and rearing, than other groups of pups. It is likely that the mother's changed behaviour, due to experience, was only perceptible to pups in a small litter. Littermate competition for the mother's attention would have been lower in small litters than in large litters.

The father's presence was also associated with faster behavioural and physical development. In the main experiment, pups reared in the presence of the father spent significantly more time, overall, rearing and eating solid food than pups without the father present. In the Split Cage experiment, pups reared with the greatest access to the father spent significantly more time overall, in exploratory behaviour and eating solid food, than other pups. In the main experiment, the body weight mean of day 30 pups was significantly greater for pups reared in the presence of the father. Faster development of offspring reared in the presence of the father has been recognized in the gerbil (Meriones unguiculatus) (Elwood & Broom 1978), the California mouse (Peromyscus californicus) (Dudley 1974a), and the laboratory mouse (Fullerton & Cowley 1971).

(ii) The Father's Caretaking Behaviour

One of the most noticeable aspects of the experimental results was the extensive and readily displayed range of caretaking behaviour provided by the father. This was most obvious in all three experiments and was more apparent in the mother's absence. The Split Cage experiment showed that the father spent as much time in the nest with the offspring as did the mother. Paternal behaviour has been noticed previously in the laboratory mouse (Leblond 1940; Noirot 1964b, Priestnall & Young 1978) and in rodents generally (Elwood 1983). The stimulation received by the pups from the father is seen to provide the means for a direct father effect on behaviour.

(iii) The Father's Proximity to Pups in a Small Litter

In the main experiment, the pups in small litters spent significantly more time, overall, in close proximity to the father, than pups in large litters. This may indicate a greater propensity on the part of the fathers to behave paternally towards single pups or small groups of pups. In the Split Litter experiment, where the father's display of caretaking behaviour was very pronounced, the father was exposed to only three pups. The FLE fathers, in the Split Cage experiment, also readily displayed caretaking behaviour if only one or a few pups came through the pup filter to the father's compartment. This contrasts with Elwood & Broom's (1978) study where gerbil fathers spent less time in contact with the pups in small litters, compared to larger litters.

(iv) The Father's Proximity to Pups Reared by a Less Experienced Mother

The main experiment showed that the pups reared by primiparous mothers spent significantly more time, overall, close to the father compared to those reared by multiparous mothers. The father may well have been compensating for inadequacies in the mother due to her inexperience. It is of interest to note that this result represents the only maternal experience main effect. Although previous work (reviewed in Chapter 1) has provided some evidence that parity influences maternal behaviour, this single maternal experience main effect is consistent with the results of Grota (1973), Hartung & Dewsbury (1979), Moltz & Robbins (1965) and Waring & Perper (1979), who found that parity had little or no effect on rodent maternal behaviour. It should be noted, however, that although there was only one maternal experience

main effect, there were several interactions involving maternal experience in my own results (see section 7, below).

(v) The Father's Effect on Pups' Exploratory Behaviour

Offspring exploratory activities were displayed more in the presence of the father than in his absence. This was the case for head-lifting and rearing in the main experiment, for sniffing and head-lifting in the Split Litter experiment, and for exploratory behaviour in the Split Cage experiment. Further, small litters in the main experiment were characterized by both a large 'proximity to father' mean score and a high 'head-lifting' mean score. Also, small-litter pups reared by a primiparous mother and with the father present, were characterized by high 'sniffing' scores. It is likely that these exploratory activities were pup responses to odours produced by the father. Adult male odours have been found to accelerate physical development in female mouse pups (Fullerton & Cowley 1971).

(vi) The Maternal Mediation Effect

In view of several interactions involving maternal experience and paternal presence, which emerged from the main experiment, it seems likely that maternal behaviour could be mediating the effects of the father. For example, pups raised by a multiparous mother and with the father present, spent significantly more time, overall, out of the nest than the other groups. The Split Cage experiment demonstrated that aspects of maternal behaviour were intensified by the presence of the father and that the consequent behaviour of pups was altered in a similar way to that noted in the main experiment. The maternal mediation route provided a second route of influence for the father to affect offspring behaviour.

(vii) Interactions Involving Maternal Experience

One of the most striking aspects of the main experiment was that the significant interactions which emerged, all involved maternal experience. This would indicate that maternal experience either mediated the effect of some of the other factors or exerted its effect by the mediation of some of the other factors. Several three-way interactions emerged, showing the importance of considering the relationship between the three social factors. When studying any one of the three factors, it would be important to either represent or control the other two factors in the experimental design. It is interesting to note that no combination of factors was excessively detrimental to mouse pup development. Behavioural development was affected by all three factors and by interactions between the factors, but no large deficits in consequent behaviour were ever observed.

2. Anomalies Within the Three Experiments

The above section has indicated the consistencies which exist between the results from the three experiments. Certain anomalies do exist, however, and it would be worthwhile to consider these.

Body weight data from the main experiment and the Split Litter experiment supported the hypothesis that the presence of the father causes the physical development of the offspring to proceed more rapidly. No clear confirmation is provided, however, by the Split Cage experiment results. In the main experiment, the mean 'day 30 body weight' for pups exposed to the father was significantly greater than that for pups reared without the father present. In the Split Litter experiment, two groups of offspring (F and NP) were

temporarily mother-deprived and, one might have predicted, likely to show depressed body weights. The results indicated, however, that pups exposed to the father (F) showed no significant depression of body weight compared to the pups exposed all of the time to the mother, (M), a similar result to that of Dudley (1974a) on the California mouse. On the basis of these results, from the main experiment and the Split Litter experiment, it might be expected that the FRI pups' mean body weight would be significantly greater than that of the NOF pups in the Split Cage experiment. Although the differences in body weight in the Split Cage experiment were in the expected direction (that is, NOF < FLE < FRI), they were not significant. This finding that paternal presence had no significant effect on offspring body weight agrees with the results of Priestnall & Young (1978) on laboratory mice, but fails to support the body weight data from earlier experiments in this project. Further study would be valuable to examine this difference between the results, and to clarify the extent to which paternal presence affects offspring body weights.

The maternal mediation of the father's influence on the offspring was featured in the Split Cage experiment. The presence or absence of the father markedly affected the display of maternal behaviour. In the presence of the father (FRI), mothers spent significantly more time, overall, in the nest and involved in nest construction; and significantly less time, overall, involved in non-caretaking activities; compared to mothers without a father present (NOF). Also, with the father present (FRI), pups spent a significantly greater period of time, overall, suckling, compared to pups without the father (FLE). It would be expected that these differences would be reflected, to a greater extent, in the main experiment results; yet here the paternal presence factor did not significantly affect either the 'proximity

to mother' or the 'suckling' mean scores. Different experimental designs may have been responsible for this anomaly, but further experimental work will be required to either confirm or refute this suspicion.

Within each experiment, the open field test results were useful for making comparisons between the effects of different treatments. Some inter-experiment comparisons were also possible; for example, pups exposed to the father in both the main experiment and the Split Litter experiment obtained a significantly larger open field defaecation mean score than pups having no access to the father. Very little consistency was apparent, however, across the three experiments in the other open field scores. The test would have been even more valuable if additional inter-experiment consistencies had been apparent. The use of the test was critically reviewed in Chapter 5 and it may be that inherent weaknesses in the test were being reflected in these results. One improvement in the way the test has been administered in my own experiments, however, would be to subject each animal to a series of tests instead of using a single test (Whimbey & Denenberg 1967).

3. Possible Routes of Influence

Within the laboratory mouse triad; involving the mother, the father and the litter; there exist a number of potential routes of influence. The mother is likely to affect the litter directly since she is a dominant influence during their development (Richards 1967). The father can affect the litter, either directly or through the mediation of the mother (the subject of Chapter 6). Several workers have suggested that the stimulus characteristics of the litter can affect maternal behaviour which, in turn, can influence the

behavioural development of the litter (for example, Barnett & Burn 1966, 1967; Richards 1966a). It is also possible for the father to influence the stimulus characteristics of the litter, which affects maternal behaviour and in turn, influences litter development (Chapter 6; Elwood & Broom 1978). The litter must also be considered an agency likely to affect the individual pups in it. Litter size effects on pup development, for example, were examined in Chapter 5. Seitz (1954) concluded that the three variables; litter size, maternal behaviour and offspring behaviour; were related and influenced each other as chains of interacting events. Fig. 7.1 shows a number of possible routes of influence; some of which have been considered in this project. Some of the routes would also constitute subject material for future research.

4. Further Research

Throughout this study, suggestions for future lines of research have been made. Further suggestions are made at this point.

Since no measurements of mouse pup behaviour or development were taken after day 30, it would be interesting to establish whether the effects noted were continued into a later stage of the subjects' lives. It might also be the case that some effects due to experimental treatments would not manifest themselves until a later period of development or stage of adult life. The recording of offspring behaviour at an older age would help to determine these matters.

Although the recording procedure was found to be satisfactory, the recording session length could now be extended in future experimentation, to establish the effect of session length on the results.

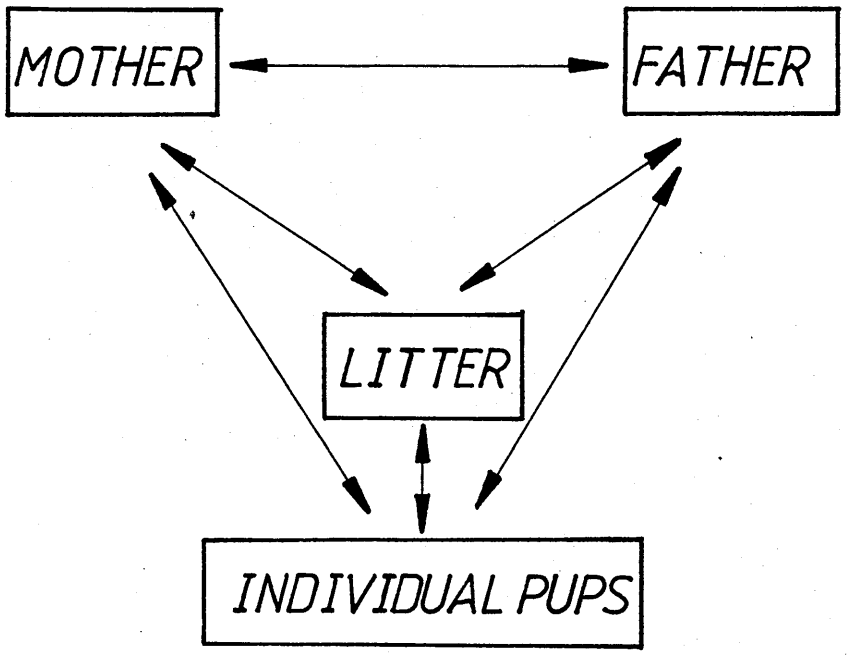


Fig. 7.1 *Possible routes of influence on behavioural development in the laboratory mouse.*

Extending the session length may also render the approach, of pooling of data over two days, unnecessary.

Since a diurnal rhythm operates in laboratory mice (Lockard 1963), it would also be valuable to investigate the effect of recording at different times during the day/night cycle. Certain treatment effects on parental and offspring behaviour were noticed in this project, where all recording was carried out at the end of the dark phase of the cycle. One could question, however, whether similar results would have been obtained if a different part of the cycle had been adopted for recording purposes.

The adult males used in my own experiments were always the biological fathers of the pups in question. Would the same results have been obtained, however, if a different male had been substituted for the father ? This raises the same question posed by Mitchell & Brandt (1969) regarding paternal behaviour in primates, that is, whether the relationship to the infant affects the display of 'paternal behaviour'. There would be a selective advantage in adult males exhibiting caretaking behaviour if the recipient pups were his own offspring. If a father did show favouritism to his own offspring, however, it would require him to be able to discriminate between his own and other offspring (Hamilton 1964). Ostermeyer & Elwood (1983) found that adult male mice were able to discriminate between their own offspring and other pups, mainly by means of olfactory cues. It would be valuable now to investigate the effect of three different types of adult male mouse on the development of a litter and on the mother's behaviour.

The three types of male would be:

1. the biological father of the pups.
2. a novel, non-biological father (that is, a male with reproductive experience, but not the father of the pups in question).
3. a naive novel adult male (that is, a male with no reproductive experience).

Some research already conducted (Smith & Simmel 1977) suggests that there would be differences due to the three different treatments.

Auditory and olfactory mechanisms have been invoked to provide an explanation for many of the results in this project. It would now be appropriate to investigate whether these mechanisms really have been responsible for the observed effects, by measuring the auditory and olfactory signals produced by the subjects.

5. Application of Results to the Wild Situation

The three social factors examined in this study were chosen because of their relevance to the wild situation (Chapter 1). It is worthwhile now to consider whether some of the results regarding the father are also applicable to the wild counterpart of the laboratory mouse. This project has closely examined the role and influence of the laboratory mouse father. If the father displayed as much caretaking behaviour in the wild, there would be a number of advantages for the wild population. Berry et al. (1973) claimed that a large number of house mice (Mus musculus) deaths in Britain are due to cold. If the father remained with the litter, insulation would be provided for the offspring and this would increase their chance of survival. Nest attendance by the father would also reduce the

caretaking load of the mother and this, in turn, could be of advantage to the pups. The mother might, as a result, show less nest aversion and also have a better opportunity to obtain food herself. Through both a direct route of influence, and through a maternal mediation route, the father's presence in my own experiments could be equated with faster pup development. Since the offspring's early developmental stage would represent a period of vulnerability in the wild, faster development would tend to aid survival.

The question remains, however, as to whether the father would display similar behaviour in the wild to that displayed in the laboratory. Brown (1953) studied mice in the laboratory which had recently descended from wild stock. Little interest was expressed by the adult males in the young, although the occasional carrying of pups was observed. Jakubowski & Terkel (1982) compared paternal behaviour in wild, recently caught house mice to that in laboratory mice. They concluded that C57 BL laboratory mice were not typical of the wild mouse in terms of parental behaviour. Since strain differences in laboratory mice cause such large differences in behaviour (Moltz 1971; Newell 1967; Steng 1971), it would be difficult to maintain that wild mice behaved in the same way as a particular strain of laboratory mouse, without first establishing that this was the case. Blizard (1971) suggested that most inbred strains have been in captivity for so long that they are likely to be genetically very different from the wild house mouse.

Another factor which would affect the display of paternal behaviour in the wild mouse, would be the existence of other adults. Since the house mouse breeds very prolifically, it is common for high population densities to be reached and for communal nesting and litter sharing to occur (own observations; Rowe 1973). It is likely that under these conditions the display of paternal caretaking behaviour would be inhibited by the proximity of several adult females (Gandleman, Pashke, Zarrow & Denenberg 1970; Lown 1980). The wild father may, therefore, only exhibit caretaking behaviour as a fail-safe measure when there are many offspring to be cared for, but few parents to provide the caretaking behaviour. Lown (1980) has concluded that the issue is not whether male mice care for their young, but rather under what circumstances the male mice would be able to maximize their reproductive success by exhibiting caretaking behaviour. The suggestion is made that the exhibition of male parental care would be more likely in situations where the male could identify his paternity. Elwood (1983) has addressed the question of the likelihood of caretaking behaviour being displayed by the rodent father in the wild. In contrast to the relative ease of laboratory mouse research, the study of wild house mice is fraught with difficulty; yet only a study of the wild mouse will establish the extent to which laboratory mouse behaviour is typical of the wild situation.

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